A STUDY ON COMMUNITY ACQUIRED BLOODSTREAM INFECTIONS, MOLECULAR CHARACTERIZATION OF RESISTANT PATHOGENS AND CORRELATION WITH INFLAMMATORY MARKERS IN A TERTIARY CARE HOSPITAL

Dissertation submitted for

M.D. MICROBIOLOGY BRANCH – 1V

DEGREE EXAMINATION

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

CHENNAI – 600 032

TAMILNADU

MAY 2019

CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON COMMUNITY ACQUIRED BLOODSTREAM INFECTIONS, MOLECULAR CHARACTERIZATION OF RESISTANT PATHOGENS AND CORRELATION WITH INFLAMMATORY MARKERS IN A TERTIARY CARE HOSPITAL"** is a bonafide record of work done by **Dr.A.PRIYADHARSHINI**, during the period of March 2017 to February 2018 under the guidance of **Prof.Dr.C.P.RAMANI, M.D.,** Professor , Institute of Microbiology , Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai - 600003, in partial fulfillment of the requirement of M.D. MICROBIOLOGY Degree Examination of The Tamilnadu Dr.M.G.R. Medical University to be held in May 2019.

Dr. R.JAYANTHI, M.D.FRCP (Glas)
DEAN,
Madras Medical College &
Rajiv Gandhi Govt. General Hospital,
Chennai – 600003.

Dr.J.EUPHRASIA LATHA, M.D.DGO
Director i/c & Professor,
Institute of Microbiology
Madras Medical College &
Rajiv Gandhi Govt. General Hospital,
Chennai – 600003.

DECLARATION

I, **Dr.A.PRIYADHARSHINI**, Post Graduate , Institute of Microbiology, Madras Medical College, solemnly declare that the dissertation titled **" A STUDY ON COMMUNITY ACQUIRED BLOODSTREAM INFECTIONS, MOLECULAR CHARACTERIZATION OF RESISTANT PATHOGENS AND CORRELATION WITH INFLAMMATORY MARKERS IN A TERTIARY CARE HOSPITAL"** is the bonafide work done by me at Institute of Microbiology, Madras Medical College under the expert guidance and supervision of **Prof.Dr.C.P.RAMANI, M.D.,** Professor, Institute of Microbiology, Madras Medical College. The dissertation is submitted to the Tamil Nadu Dr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch IV) in Microbiology.

Place: Chennai **DR.A.PRIYADHARSHINI**

Date:

Signature of the Guide

Prof.Dr.C.P.RAMANI, M.D.,
Professor,
Institute of Microbiology
Madras Medical College, Chennai - 600 003.

ACKNOWLEDGEMENT

I am extremely thankful to **DR.R.JAYANTHI, M.D.FRCP (Glas),** Dean, Rajiv Gandhi Government General Hospital & Madras Medical College, Chennai-3, for giving me permission to commence this dissertation work and use the resources of this institution.

I am immensely grateful to **Prof.DR.J.EUPHRASIA LATHA, M.D.DGO, Director** in charge & professor Institute of Microbiology, for her support and encouragement given throughout this study.

I would like to express my sincere gratitude to former professor, **DR.MANGALA ADISESH M.D.,** Institute of Microbiology for her valuable advice and support given during this work.

My deepest gratitude and profound heartfelt thanks to my guide, **Prof. DR.C.P.RAMANI,M.D.,** professor, Institute of Microbiology, for her vital support and in guiding me in right path. Her encouragement and motivation made it possible to achieve the goal.

My sincere thanks to former Director, Institute of Internal Medicine, **Prof. DR.S.MAYIL VAHANAN, M.D., (Retd),** former Director, Institute of Surgery, **Prof.DR.A.RAJENDRAN, M.S., (Retd),** former Head of the department of Geriatric Medicine, **Prof.DR.S.SIVA KUMAR, M.D. (GERIATRICS), D.T.R.D** and also to the current in charge of Intensive Medical Care Unit, **Prof.DR.S.RAGUNANTHANAN, M.D.,** for allowing me to collect samples from the patients for this study.

I also take this opportunity to thank **Prof.DR.BHARATHI VIDHYA JAYANTHI, M.D.,** Director and professor, Institute of Pathology, Madras Medical College for her kind help to carry out this work.

My sincere thanks to all my professors, **DR.S.THASNEEM BANU, M.D., DR.U.UMADEVI, M.D.,** and **DR.R.VANAJA, M.D.,** for their support during this study.

My profound thanks to **DR.S.MEENAKSHI,D.D,M.D.(Micro),** Associate professor(Imm.), Department of Rheumatology and **DR.D.THERESEMARY, DCH,M.D.(Micro),** Associate professor, Dharmapuri Government Medical College for their valuable advice and timely help in carrying out this study.

I would also like to thank my co-guide, **DR.J.PADMA KUMARI, M.D.,** for her constant support and guidance in this study.

I owe my gratitude to my former co-guide, **DR.B.NATESAN, M.D. DLO.,** for his support, motivation and encouragement given during this study.

I wish to thank all my Assistant professors, **DR.N.LAKSHMIPRIYA, M.D.D.CH., DR.K.G.VENKATESH, M.D., DR.R.KESAVAN, M.D., DR.P.SHANMUGA PRIYA, M.D., DR.C.NITHYA, M.D., DR.S.VINOTHA, M.D., DR.B.GOMATHI MANJU, M.D.,** for their support.

I also wish to express my heartfelt thanks to our former Assistant professors, **DR.R.DEEPA, M.D., DR.K.USHA KRISHNAN, M.D., DR.C.S.SRIPRIYA, M.D., DR.DAVID AGATHA, M.D., DR.N.RATHNA PRIYA, M.D.,** for their encouragement and support.

My sincere thanks to **INDIAN COUNCIL OF MEDICAL RESEARCH,** for their grants provided for this dissertation work, without which it would be impossible to reach the goal.

Also, I would like to thank the **INSTITUTIONAL ETHICS COMMITTEE,** Madras Medical College for approving my study.

I would like to extend my thanks to all my postgraduate colleagues and technicians for their kindness and support given throughout this study.

I am also thankful to the postgraduates and staffs of Medicine, Surgery and Intensive Medical Care Units for their help given during this study.

Special thanks to my mother, **MRS.RAJESWARI AGHORAM,** for her constant support, motivation, encouragement, for being with me in all my hardships and I also thank my sister and Brother-in-law for their wonderful support and advice.

Above all I thank the almighty in helping me to carry out this study smoothly.

I am extremely thankful to all the patients and their family members who had cooperated well to carry out this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF COLOUR PLATES

Sl.No.	TITLE
19	CRP detection-latex agglutination test-picture2
20	IL-6 picture 1
21	IL-6 picture2
22	IL-6 picture3
23	ELISA washer
24	IL-6 Graph plotted on a semi log paper
25	bla CTX-M gene detection (ESBL)
26	bla TEM gene detection (ESBL)
27	mecA gene detection(MRSA)

URKUND

Urkund Analysis Result

Analysed Document: DR PRIYADAR MICRO.docx (D42404450)
Submitted: 10/11/2018 8:34:00 AM
Submitted By: priyammc9@gmail.com
Significance: 2 %

Sources included in the report:

http://epublications.uef.fi/pub/urn_isbn_978-951-27-1389-9/
http://www.doria.fi/handle/10024/95530
https://sepsistrust.org/wp-content/uploads/2018/06/Sepsis_Manual_2017_web_download.pdf
https://www.world-sepsis-day.org/news/2018/1/9/who-statement-on-maternal-sepsis

Instances where selected sources appear:

7

CERTIFICATE II

This is to certify that this dissertation work titled **" A STUDY ON COMMUNITY ACQUIRED BLOODSTREAM INFECTIONS, MOLECULAR CHARACTERIZATION OF RESISTANT PATHOGENS AND CORRELATION WITH INFLAMMATORY MARKERS IN A TERTIARY CARE HOSPITAL "** of the candidate **Dr.A.PRIYADHARSHINI** with registration number **201614008** for the award of M.D.,Degree in the branch of Microbiology. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows two percentages **(2%)** of plagiarism in the dissertation.

Guide & Supervisor sign with seal

Introduction

INTRODUCTION

- Bloodstream infections are one of the serious and life-threatening clinical conditions leading to deleterious consequences with mortality rate ranging from 20-40 % [1, 2]. Hence, needs immediate attention and treatment.
- Advances in blood culture techniques have resulted in efficient and reliable methodologies for the detection of causative pathogens.
- Bloodstream infections are classified traditionally as nosocomial and community acquired bloodstream infections [3, 4].
- Community acquired bloodstream infections refers to the infections detected within 48 hours of hospitalization, showing positive blood culture and develops spontaneously without an association with any prior medical interventions [5].
- Community acquired bloodstream infections are becoming a major health problem in the upcoming years due to the emergence of antimicrobial resistant organisms in community settings as causative agents like, ESBL producing Enterobacteriaceae, Methicillin resistant Staphylococcus aureus etc...[6]
- Antimicrobial resistant strains once confined to hospital settings are now a potential threat in the community too.
- Rapid detection of antimicrobial resistant strain is highly essential, as they are associated with increased mortality and morbidity and due to their high

propensity to spread and able to cause a serious threat to public health concern.

- Phenotypic characterization of microorganisms helps in identification of causative agents of infectious diseases.
- Molecular characterization of resistant pathogens aids in tracking the spread of antimicrobial resistance in community and hospital settings.
- Sepsis, since 1991, defined as the combination of infection with 2 or more features of systemic inflammatory response syndrome(SIRS): [7]

    1) Body temperature $>38^0$c or $<36^0$c
    2) Heart rate >90 beats/min
    3) Respiratory rate >20 breaths/min
    4) White blood cell count $>12x10^9$/L or $<4x10^9$/L

- Additional criteria for sepsis – altered mental status, edema, hyperglycemia in the absence of diabetes and elevated biomarkers of sepsis such as C - reactive protein, interleukins etc.., [8]
- Since 2014, sepsis is defined as a life threatening organ dysfunction caused by a dysregulated host response to infection in addition to above parameters.[9]

- C-reactive protein is an acute phase reactant protein whose synthesis in liver is upregulated by Interleukin-6.It is a well established biomarker of infection and

inflammation. Commonly used to screen for early onset sepsis, as its sensitivity is found to be very high in this setting [10, 11].

- Interleukin-6 is an immune protein in the hematopoeitins family. It is a monomer of 184 amino acids produced by T-cells, macrophages and endothelial cells found on a single gene located at 7p21.It is released in response to infections, burns, trauma, neoplasia etc..
- Interleukin-6 meets one of the desired attributes of an ideal biomarker of sepsis because it is capable of identifying those patients with sepsis, who are at increased risk of developing severe sepsis and who therefore needs supportive therapy.[12]
- Hence, inflammatory markers have a diagnostic and prognostic value in detection of sepsis.

Aims &objectives

AIMS AND OBJECTIVES

1. To identify the clinical profile of patients with community acquired blood stream infections.
2. To detect the pathogens causing community acquired blood stream infections and their antimicrobial susceptibility pattern.
3. Correlation of inflammatory markers with blood culture positivity.
4. To assess the prognosis, severity and outcome of disease with the inflammatory markers.
5. To perform the molecular characterization of resistant pathogens.

Review of Literature

REVIEW OF LITERATURE

Sepsis is a devastating, life threatening medical emergency, which needs immediate attention and treatment.

HISTORY OF SEPSIS-

The word "sepsis" originated from an ancient Greek word [σηψις] which means "decomposition of animal, vegetable or organic matter in the presence of bacteria".[13]

The word "sepsis" got its origin during Hippocrates period (ca.460-370BC) who was a great physician and philosopher, known in medical field as the "Father of modern medicine"[14]

Ignaz Semmelweis, (1818-1865), further developed his modern views on sepsis. He was an obstetrician and has done major work on "Etiology, terminus and prophylaxis of puerperal fever". Also detected childbed fever was caused by "decomposed animal matter which entered the bloodstream" and he also succeeded in reducing the mortality rate by introducing a new system, hand washing with a chlorinated lime solution before every gynecological examination. But his works were not accepted and were not well appreciated and practiced at that period of time.

Louis Pasteur, a French chemist (1822-1895) discovered the fact that tiny single celled organisms which he called bacteria or microbes caused putrefaction, in other words “sepsis”.

Joseph Lister (1827-1912), a surgeon found that, in his hospital about 50% of patients following amputation procedure died of sepsis. Then he correlated Semmelweis findings, Pasteur’s views and deaths which occurred in his hospital, and as a result he introduced his “antiseptic method” successfully by examining the effects of skin and instrument disinfection with carbolic acid.

It was Hugo Schottmuller (1867-1936), student of H. Lennhartz, a German physician in 1914 gave a modern definition for sepsis as follows:

“Sepsis is present if there is a focus from which pathogenic bacteria invade the bloodstream either constantly or periodically, so as to cause the subjective and objective symptoms”.

From above definition, it was understood for the first time, that source of infection came into focus as a cause of sepsis.

During the pre-antibiotic time, mortality rates from sepsis were very high. Only after introduction of effective antibiotics after worldwarII, mortality rates due to sepsis reduced. With progress in technology, Intensive medical care units started to develop and sepsis patients became the main fractions who were dealt with, under these units.

In 1989, US American ICU specialist, Roger C. Bone (1941-1997), introduced the concept of sepsis syndrome and gave a new definition for sepsis, which is still valid until today. “Sepsis is defined as a systemic response to a suspected or documented infection with at least one organ dysfunction”.[15, 16]

Definition of Sepsis:

The old and new definitions and concepts of sepsis is as follows-

Sepsis –definition1

1991 Consensus Conference convened by the Society of Critical Care Medicine and the American College of chest physician, defined[17]

Sepsis as the systemic response to infection and as a result of infection manifests by two or more of SIRS criteria.

Severe sepsis is sepsis associated with organ dysfunction, hypo perfusion or hypotension.

Septic Shock is sepsis with hypotension despite adequate fluid resuscitation along with the presence of perfusion abnormalities.

SIRS criteria

(a) Temperature > 38℃ or <36℃

(b) Heart rate >90 beats per minute

(c) Respiratory rate >20 breaths per minute or $paco_2$ <32mmHg

(d) White Blood Cell count >12,000/cumm, <4,000/cumm, or 10% immature (band) forms.

Sepsis- definition2

2001 Consensus Conference sponsored by the Society of Critical Care Medicine, the European society of Intensive Care Medicine, the American college of clinical pharmacy, the American thoracic society and the Surgical Infection Society gave a detailed diagnostic criteria for sepsis as follows-[17]

a) Infection – suspected or documented.

b) General parameters-

(1) Fever >38℃ or <36℃

(2) Heart rate >90 beats/minute

(3) Respiratory rate >30 breaths per minute

(4) Altered Mental Status

(5) Edema

(6) Hyperglycemia in the absence of diabetes.

c) Inflammatory parameters-

(1) white blood cell count >12,000/ L or <4,000/ L

(2) Plasma C – reactive protein >2SD above the normal value

(3) Plasma procalcitonin >2SD above the normal value

d) Hemodynamic parameters-

(1) Hypotension (systolic blood pressure 90mmHg)

(2) Mixed venous oxygen saturation >70%

e) Organ dysfunction parameters-

(1) Acute oliguria (urine output <0.5ml/kg/hr for at least 2hrs and increase in Sr.creatinine > 0.5mg/dl)

(2) Arterial Hypoxemia (pao_2/Fio_2<300)

(3) Coagulation abnormalities (INR >1.5 or aPTT >60s)

(4) Thrombocytopenia (platelet count <1,00,000/µl)

(5) Hyperbilirubinemia (plasma total bilirubin >4mg/dl)

(f) Tissue perfusion parameters-

(1) Hyperlactatemia (>3mmol/l)

(2) Decreased capillary refill.

Sepsis –definition3

In 2014, the European society of Intensive Care Medicine and the Society of Critical Care Medicine organized a task force to update the definitions and clinical criteria of sepsis and gave a new definition. Henceforth, the results were published in February 2016 issue of JAMA, the Journal of American Medical Association.

Also, in this conference reduced the clinical stages from 4 to 2 (singer et al, 2016) (two stages- sepsis, septic shock)

Defined **sepsis** as a life threatening organ dysfunction caused by a dysregulated host response to infection.[18, 19]

This new definition is being widely used in recent clinical practice.

Septic shock is sepsis with underlying circulatory and metabolic abnormalities.

Screening for sepsis-

q SOFA scoring system (quick sequential organ failure assessment) [20]

It includes:-

(1) Altered Mental Status (GCS score <15)

(2) Systolic blood pressure < or = 100mmHg

(3) Respiratory rate > or = 22breaths per minute

SOFA scoring system is used to determine the sepsis related organ dysfunction. It calculates the level of dysfunction in 5 systems namely respiratory, cardiovascular, coagulation, renal and neurological systems. It is calculated at admission in every 24hrs.

According to sepsis consensus conference 2016 definition, sepsis can be diagnosed, if there is suspected or documented infection and an acute increase of >or= 2 SOFA points.[21]

Blood Stream Infections-

Blood stream infections are infections caused by the presence of viable bacterial or fungal microorganism in the blood stream showing positivity of one or more blood cultures and also accompanied by an inflammatory response characterized by alteration of clinical, laboratory and hemodynamic parameters. Blood stream Infections can be either primary or secondary infections.

Primary blood stream infection is an infection where there is no identifiable source.

Whereas **secondary blood stream infection** is an infection seeded from a site specific infection at another body site. In other words there is an identifiable source such as urinary tract, respiratory tract, genitourinary tract, intraabdominal infections etc...

Blood stream infections are traditionally classified as **nosocomial** or **community onset blood stream infections.**

Community onset blood stream infections are those identified less than 48hrs of hospital admission and may be further sub-classified as **health care associated** and **community acquired blood infections.**

Health care associated infections are those infections detected within 48hrs of hospital admission, showing positive blood culture and have to fulfill any one of the following criteria-

(a) Received intravenous medical therapy in any form or wound care in 30

(b) days before the blood stream infection either at home or in any health care facilities.

(c) Visited a hospital or haemodialysis clinic in 30 days before blood stream infection onset.

(d) Being hospitalized in health care facility for two days or more within 90 days before onset of blood stream infection.

(e) Resided in a nursing home or long term health care facility.

Community acquired blood stream infections are defined as infections detected within 48 hrs of hospital admission, showing positive blood culture and those who do not fit into the criteria of health care associated infection as mentioned above.[22]

Risk Factors:

The following are the risk factors associated in lesser frequency compared to nosocomial infections [23, 24]

a. Demographics [25, 26]

- Elderly age group
- Males are at increased risk than females

b. Underlying diseases

- Diabetes mellitus
- Hypertension
- Chronic liver disease

- Chronic kidney disease
- Chronic obstructive pulmonary disease
- Malignancy
- Stroke

c. Co-Morbid conditions

- Smoking
- Alcohol uptake
- Neutropenia
- Use of steroids
- History of transplantation

Sources of infections [1]

- Urinary tract
- Respiratory tract
- Intra abdominal infections
- Biliary tract
- Skin and soft tissue infections
- Unknown sources

Microbiology

Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae are the commonest causative agents [27,28]

Other less frequent causative agents

- klebsiella pneumoniae
- Salmonella spp.
- Proteus spp.
- Pseudomonas aeruginosa
- Enterobacter spp.
- Serratia spp.
- Citrobacter spp.

Other rare etiologies-

- Morganella spp.
- Providencia spp.

Other Gram positive cocci-

- Streptococcus pyogenes
- Streptococcus agalactiae
- Enterococcus faecalis

These are rarely isolated or restricted to particular geographical area only. E.g. Brucella spp, Burkholderia pseudomallei.[29]

Candida spp-

- Occasional cause of community onset blood stream infections and are typically associated with health care associated infections.

CONS (Coagulase Negative Staphylococcus aureus)-

- Predominantly related to health care associated infections and less frequently isolated in community onset blood stream infections.

Pathogenesis-

Bloodstream infection is a highly complex and life threatening clinical entity in which pathogenesis is multifactorial.

Bacteraemia results from imbalances in the complex interplay between the invading microorganism and the defense mechanisms of the host.

Following are the host defense mechanisms-[30]

a) Innate immune mechanisms-

Innate immune cells generally recognize the microorganisms by sensing common microbial structures known as pathogen associated molecular patterns(PAMPS) eg-lipopolysaccharides.

Receptors on surface of immune cells called pattern recognition receptors recognize and attach these PAMPS.

Toll-like receptor is an important family of pattern recognition receptors (PRRs) and has a major role in host defense mechanism against microorganisms.

Attachment of PRRs to their ligands leads to activation of transcription factors e.g.- nuclear factor kappa β, that in turn leads to the production of proinflammatory cytokines like TNFα,IL1,IL2,IL6,IL8,IFγ.

These are the general mechanism of innate immune system in host defense.

Components of innate immune system which renders the function-

Barriers, innate immune cells.

Barriers-first barrier to pathogen invasion is skin and mucous membrane.

Microbes commonly enter the body through skin and mucosa of gastrointestinal tract and respiratory tract. Following which antigen presenting cells residing in the epithelium mainly dendritic cells, capture these microbial antigens and present them to naive T-lymphocytes which then differentiate to effector cells and then destroy these microbes. Hence, loss of skin and mucosal barrier leads to microbial invasion and infection.

Another potent and important barrier to pathogen invasion is the complement system.

Following bacterial infection, complement gets activated through classical, alternative or mannose binding lectin pathway and helps in pathogen destruction.

Any defect in any of these pathways leads to invasive infections.

Cellular innate immune response- neutrophils are the most important cellular host defense against invading pathogens.

They rapidly migrate from blood to the site of infection which is mediated by chemo attractants like IL8, LTB4 etc... and then recognize and phagocytose the invading microorganisms.

Microorganisms contained in the phagosomes are killed further by NADPH oxidase dependent and myeloperoxidase dependent reactive oxygen species or by antimicrobial peptides of cytoplasmic granules.

Hence, neutrophils play a major role in control and in the clearance of invading microorganisms.

Congenital disorders or acquired deficiencies in number or functions of neutrophils predisposes to invasive infections both by Gram positive and Gram negative bacteria and also by fungi.

Other cells involved in phagocytosis include- tissue macrophages, dendritic cells, and natural killer cells.

Role of natural killer cells in bacteraemia and sepsis is significant. Increased apoptosis of natural killer cells takes place during transition from sepsis to severe sepsis or septic shock in patients with bloodstream infections.

b) Adaptive immune response-

Adaptive immune response stimulated later during infection process includes B cell and T cell responses.

T cell response- presentation of exogenous antigens or microbes to T-lymphocytes by antigen presenting cells via MHC-I or II dependent manner which in turn leads to production of pro-inflammatory cytokines critical for the clearance of invading microorganisms.

B cell response- results in production of antibodies against specific antigenic components of a certain pathogen. Antibodies interact with the pathogen, hence neutralizing their effect and limiting microbial infectivity.

Hence, defect in either T or B cell leads to detoriation of adaptive immune response and flaring up of infection and septicaemia.

Liver and spleen also plays a major role in host defense. Liver function as filters of bacteria from bloodstream and spleen, a major site of antibody production.

Hence any defect or damage to these organs will affect the host defense mechanism leading to microbial invasion and infections.

In pathophysiology of sepsis, in addition to above mechanisms, there occurs an abnormality in the coagulation system which causes local disturbances in hemostasis.

Virchow's classic triad includes- changes in coagulability, endothelial cell injury, and abnormal blood flow.

In sepsis, all 3 alterations are seen which results in reduced blood flow to vital organs and hence poor tissue perfusion to vital organs with resulting cytopathic hypoxia leads to multiorgan failure and dysfunction.[31]

The above changes in pathophysiology of sepsis is explained further as follows-

At the cellular level, sepsis is characterized by changes in endothelium, alterations in coagulation process and in blood flow.

These changes are initiated by the cellular release of pro- inflammatory substances such as cytokines, in response to the presence of infectious microorganisms.

These pro- inflammatory cytokines are short- lived regulatory proteins which interact with the endothelial cells causing injury to the endothelium and death of endothelial cells by apoptosis.

These interactions between pro- inflammatory substances and endothelial cells results in activation of coagulation factors.

Endothelial damage leads to increased vascular permeability and leaky blood vessels.

Hence, the fluids and microorganisms escape into the surrounding tissues leading to edema.

In lungs, it results in pulmonary edema. Cytokines also causes vasodilatation of the blood vessels, causing a decrease in blood pressure.

This endothelial damage resulting from inflammatory response explains the systemic nature of sepsis.

Diagnosis-

a) Blood culture-

Even though several advanced and newer techniques are upcoming, blood culture still remains the gold standard in diagnosis of bloodstream infections.

Indications of blood culture-

Fever with chills or of unknown origin

Septic shock

Leucocytosis

Suspected endocarditis

Pneumonia

Meningitis

Peritonitis

Timing of blood culture specimen collection- prior to antimicrobial therapy initiation[32]

Volume of blood sample collected definitely has an impact on the yield - The higher the volume of blood cultured, the higher will be the yield and the rate of detection of bloodstream infections.[33, 34]

Incubation time- incubated for a maximum duration of 5-7days.

b) Biomarkers of sepsis-

1) CRP (C-reactive protein)-

It is a significant marker of both inflammation and infection

It is an acute phase reactant protein belongs to pentraxin family of proteins. Members of these family posses a cyclic pentamer composed of five identical non-glycosylated polypeptide subunits which are non-covalently bound and well organized as a stable discoid like structure.

It is synthesised in liver by hepatocytes, chiefly in response to IL6 and there is often a good correlation exists between CRP and IL6.

It is also produced in small amounts by non-hepatic cells such as neurons, monocytes, atherosclerotic plaques, kupffer cells, lymphocytes, alveolar macrophages, kidneys.

It has a half life of 19hrs.[35]

CRP secretion begins within 4-6hrs of exposure to a stimulus and peaks at 36-48hrs.

Serum concentration of CRP in normal healthy adult population ranges between 0.3-1.7mg/l and is below 10mg/l.

Mild changes in CRP levels-vigorous exercise, heat stroke etc...

Marked rise in CRP levels-acute bacterial infections, systemic fungal infections and in other invasive infections.

Main role of CRP-is to provide clearance of inappropriate materials of extrinsic origin such as microorganisms and their products or the autologous products of cell damage and death from plasma.

Methods of measurement of CRP-

Latex agglutination test- initially qualitative measurement done followed by semi-quantitative measurement.

Several immunological methods- enzyme immunoassay, immune-turbidimetry, nephelometry.

Applications- it has wide applications in the diagnosis of infectious diseases and also as a prognostic indicator not only in adults but also used as a marker of early onset sepsis in paediatric population as well.

It's currently widely used in rheumatology and transplantation medicine. Its application in the field of cardiology is also increasing especially in coronary artery disease in the form of hs-CRP (highly selective CRP) [36] which is being used as a tool for cardiac risk evaluation and as a prognostic factor in acute coronary syndrome.

2) IL-6 (Interleukin-6)-

- It is a multifunctional cytokine which belongs to the IL6 family.
- Human IL-6 consists of 184 amino acids with 2 potential N-glycosylation sites and 4 cysteine residues.
- It is produced by various cells such as T cells, B cells, monocytes, endothelial cells, fibroblasts, mesangial cells, keratinocytes, several tumour cells, astrocytes, bone marrow stroma cells.
- IL6 receptors exists in 2 forms- a soluble IL6 receptor(s IL6R) and a membrane bound IL6 receptor (m IL6R).
- IL6 Trans -signalling is mediated through s IL6R by activating cells that express gp130.
- Classic signalling is mediated through m IL6R.

Mechanism of action-

- IL6 on binding to IL6R results in either homo or heterodimerization of gp130 subunit and leads to the formation of IL6/gp130 complex.
- This complex in turn activates Janus tyrosine kinase /signal transducer and activator of transcription (STAT) pathway (JAK-STAT pathway).
- IL6 has various pleiotropic functions, [37] of which salient ones are as follows-
    - B cells- Ig production
    - T cells- proliferation and differentiation

- Hematopoietic progenitor cells- enhancement of multipotential hematopoietic colony formation.
- Hepatocytes-acute-phase protein synthesis.
- Blood vessels- proliferation of vascular smooth muscle cells.
- Bone metabolism- stimulation of osteoclast formation and induction of bone resorption.
- Heart muscle cells- negative ionotropic effect on heart.
- On placenta- secretion of chorionic gonadotropin from trophoblasts.

IL6 and acute phase response-

Acute phase response is the response exhibited by the organism to disturbances in homeostasis resulting from infection, tissue damage, immunological disorders or any neoplastic growth, trauma or surgery.[38]

IL6 and CRP-

IL6 is the main mediator stimulating CRP production, but other cytokines like IL1 and TNF are also involved.

Antimicrobial resistant organisms in community settings-

Antimicrobial resistant organisms such as extended-spectrum β-lactamases producing Enterobacteriaceae especially E.coli, Methicillin-resistant Staphylococcus aureus have emerged as an important etiological agents of community-onset bloodstream infections.

During late 1990's and 2000 ESBL producing Enterobacteriaceae mostly E.coli strains have been identified predominantly from the community.

ESBL producing E.coli has gained worldwide recognition as a significant group of community acquired pathogen.[6]

Most patients with community onset infections caused by these ESBL producing E.coli presents with urinary tract infections, some with intraabdominal infections and concomitant bacteraemia.

Since mid 1990's prevalence of community acquired methicillin-resistant Staphylococcus aureus (CA-MRSA) infections also started increasing. Recently its prevalence is around 39%.[39]

An awareness of these strains by the clinicians and enhanced testing by the laboratories, including molecular characterization and surveillance is required in order to frame strategies to curtail these resistant strains from spreading within the community and to limit their introduction into hospitals and to reduce treatment failures.

Epidemiology-

There is an increase in incidence rate of bloodstream infections which is evident from a study done between 2008 -2014, about 14% increase from 174 in 2008 to 197 per 1,00, 000 population in 2014. [40]

Increase in number of bloodstream infections observed among individuals >= 65yrs in another study, from 119 to 144 episodes per 100 000 population.

Significant increase in bloodstream infections observed in emergency departments in a study, from 58 to 79 per 100 000 population.

Incidence of sepsis and septic shock was around 101.8 and 19.3 per 100 000 persons-years respectively in 2015.[41]

In United States, incidence of severe sepsis- 300 cases per 100 000 population.[42]

Bacteraemia is a serious condition associated with a case fatality rate of 20-30%.[1, 2]

Overall mortality of the patients with community acquired bloodstream infections ranges between 39-42%

Prevalence of community acquired bloodstream infections ranges between 7-9%.

Overall mortality of sepsis syndrome ranges between- 20-60%[43]

Septic shock associated with mortality of nearly 50%.[44]

According to CDC data reports, more than 1.5 million people get sepsis each year in the U.S.

About 2, 50,000 Americans die from sepsis each year. 1 in 3 patients who die in a hospital has sepsis.[45]

Global mortality rate in cases with septic shock and severe sepsis are 44.5% and 34.4% respectively.[46]

Prognostic factors and outcome-

Patients admitted in ICU with community acquired bacteraemia presents with a crude mortality near to 40% compared with a mortality of 18% in bacteraemia patients admitted in general wards.

Prognostic factors responsible for outcome of patient-

- Age
- Severity of patient's underlying disease
- Source of infection
- Type of organism involved
- Complications and associated co-morbidities
- Appropriateness of empiric antimicrobial treatment
- Associated secondary infections.
- Mortality among patients with inappropriate empiric antibiotic treatment is around >70%.[47]

Complications-

1. Septic shock
2. End organ dysfunctions-
    a) Lungs-acute respiratory distress syndrome
    b) Brain-encephalopathy
    c) Liver failure
    d) renal failure
    e) Heart failure

Prevention of sepsis-

As per CDC recommendations, the health care professionals should take following measures to prevent sepsis-[48]

1. Prevention of infection by following infection control practices like proper hand washing protocols & ensure that patient gets recommended vaccines. (E.g. influenza, pneumococcal).
2. To educate patients & their families on the need to prevent infections.
3. If sepsis is suspected, need to act faster by ordering tests which will detect sepsis early. To start appropriate antibiotics and recommended medical care immediately. Also, to document all aspects of the antibiotic regimen.

Materials & Methods

MATERIALS AND METHODS

Study design **:** Cross-sectional study.

Study centre **:** Institute of Microbiology, in collaboration with Institute of Internal Medicine and Surgery, RGGGH, MMC, Chennai.

Duration of study **:** 1 year (from March 2017- February 2018)

Sample size **:** 150

Study population:

Febrile adult patients with sepsis admitted within 48hrs in Medicine wards, Intensive Care Unit and Surgical wards at Rajiv Gandhi Government General Hospital.

ETHICAL CONSIDERATION-

Ethical clearance was obtained from Institutional Ethics Committee before starting the study. Informed consent was obtained from the study population. Patients those who where satisfying the inclusion criteria were included in this study. Study population was interviewed by a structured questionnaire.

INCLUSION CRITERIA:

1. Febrile patients in the age group >18 years.
2. Patients with sepsis admitted to hospital within 48hrs.

EXCLUSION CRITERIA:

1. Patients in the age group <18 years.
2. Patients with signs and symptoms of sepsis developed after 48hrs of hospitalization.
3. Patients already on antibiotic therapy.

METHODOLOGY

a) BLOOD COLLECTION PROCEDURE-

Under strict aseptic precautions, the skin over the venipuncture site was cleansed in a circle approximately 5cm in diameter with 70% isopropyl alcohol rubbing vigorously and allowed to air dry.[49] Then applied 2% povidone-iodine starting in the centre of the circle then moved towards periphery and allowed it to dry on the skin for at least one minute. The needle was then inserted into the selected appropriate peripheral vein and about 5ml blood sample was collected for processing and culture identification.

b) SAMPLE PROCESSING-

The blood sample was inoculated into brain heart infusion broth and incubated overnight at 37℃. These inoculated bottles were examined after 24hrs for any turbidity, discoloration or clotting. The first subculture was done on to MacConkey agar, blood agar, chocolate agar and sabouraud dextrose agar and incubated overnight at 37℃ and also a Gram film was performed. These bottles were again incubated and checked for turbidity twice daily. A final subculture was done on seventh day on the same medias mentioned above and also a Gram film

was performed. The blood culture was considered negative if no growth occurred even on the seventh day of subculture i.e. final subculture. Any growth which occurred during this seven day period of incubation was identified based on Gram stain, colony morphology and various biochemical reactions.

Direct Gram Film-

Gram film was examined as soon as the subcultures had been setup and interpreted.

Examination of subcultures-

After 24 hrs of incubation, the subcultured plates were examined. If growth was found, the colony morphology was recorded. Then, further the colonies were identified by –

a) Gram stain- to identify Gram positive or Gram negative organisms.
b) Motility test by hanging drop method- to identify whether the organism is motile or non-motile.
c) Other preliminary tests- catalase test, oxidase test were also performed
d) Organisms further speciated based on biochemical reactions- IMVic reactions (includes- indole test, methyl red test, Voges-proskauer test and citrate test), urease test, triple sugar iron agar test medium and the sugar fermentation medium.

CULTURE IDENTIFICATION-

a) Staphylococcus aureus-

Nutrient agar- Showed 1 to 3mm diameter, circular, smooth, low convex, glistening densely opaque colonies with golden yellow pigmentation.

Blood agar- Colonies were surrounded by a narrow zone of beta hemolysis.

MacConkey agar- Colonies were pink and were smaller in size.

Organisms confirmed with biochemical reactions.

1. **Slide coagulase test-** in a clean grease free glass slide, a colony of test organism was emulsified in a drop of normal saline to form a smooth milky suspension. To this suspension, a drop of plasma was added and the slide was rotated back and forth. Coarse clumping of the suspension visible to the naked eye within 10 seconds was considered positive and absence of clumping as negative. The test was performed with appropriate controls.
2. **Tube coagulase test-** to 0.5ml of diluted plasma (1 in 6 dilutions in 0.85% Nacl), a colony of Staphylococcus was emulsified in a tube and incubated at 37℃ for 4hrs preferably in a water-bath along with appropriate controls. Examined the tubes at 1, 2 and 4hrs for clot formation by tilting the tube through 90^0. Any degree of clot formation was considered positive. If the plasma remained as wholly liquid, it was considered as negative.[50]

   Both slide and tube coagulase tests were found positive.

BIOCHEMICAL REACTIONS-

Indole test	Negative
Methyl red test	Positive
Voges-proskauer test	Positive
Mannitol	Fermented
Urease test	Positive

Hence, with above findings, identified as Staphylococcus aureus.

Escherichia coli-

Nutrient agar- Colonies were 1-3mm diameter, circular, low convex, smooth colonies with no pigmentation or odour.

Blood agar- Greyish white colonies with hemolysis.

MacConkey agar- Flat, pink, lactose fermenting colonies.

BIOCHEMICAL REACTIONS-

Indole test	Positive
Methyl red test	Positive
Voges proskauer test	Negative
Citrate	Negative
Glucose	Fermented with acid & gas
Lactose	Fermented with acid & gas
Sucrose	Not fermented
Maltose	Fermented with acid & gas
Mannitol	Fermented with acid & gas
TSI	Acid slant/Acid butt with gas & no H_2S
Urease test	Negative

Pseudomonas aeruginosa[51]

Nutrient agar- colonies were large, low convex with serrated margins with bluish green pigmentation and earthy odour.

Blood agar- diffuse hemolysis was present.

MacConkey agar- Non-lactose fermenting colonies.

Indole test	negative
Methyl red test	negative
Voges- proskauer test	negative
Citrate	utilized
Glucose	Oxidatively utilized
Lactose	Oxidatively utilized
Mannitol	Not fermented
Xylose	Oxidatively utilized
TSI	Alkaline slant/no change in the butt
OF test	Oxidative pattern
Urease test	positive

Acinetobacter baumannii [51]

Blood agar- smooth, opaque, raised colonies

MacConkey agar- non-lactose fermenting colonies with a faintly pink tint.

Indole test	negative
Citrate	utilized
Glucose	Not fermented
TSI	Alkaline slant/no change in the butt
10% OF lactose	Oxidatively utilized
Urease test	negative
Growth at 42℃	Good growth

Antimicrobial susceptibility testing for isolated organisms done on Mueller Hinton agar plate by Kirby- Bauer disk diffusion method under CLSI guidelines.

PROCEDURE FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING-

1) **Preparation of turbidity standard equivalent to McFarland 0.5:**
   a) 1% v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of distilled water and was mixed well.
   b) 1% w/v solution of barium chloride was prepared by dissolving 0.5 g of dihydrate barium chloride in 50ml of distilled water and was mixed well.
   c) 0.6 ml of barium chloride solution was added to 99.4 ml of sulphuric acid solution and was mixed well. This mixture was transferred into a screw capped tube which could be used during inoculum preparation.

2) **METHOD-**
   a) Using a sterile loop, 3-5 well- isolated colonies of test organism, of similar appearance were inoculated into 3-4ml of nutrient broth and emulsified it.
   b) The turbidity of the above suspension was matched with the turbidity standard equivalent to 0.5 under good light and viewed against a printed card or sheet of paper.
   c) Within 15 minutes after adjusting the turbidity of the inoculum, a sterile cotton swab was dipped into the adjusted suspension, rotated several times in it and then pressed firmly on the wall of the tube above the fluid level to remove excess inoculum from the swab.

d) Using this swab streaking was done evenly over the surface of Mueller Hinton agar medium in three directions, by rotating the plate approximately 60^0, to ensure for even distribution of the inoculum.

e) 3-5 minutes waited for the surface of the agar to dry.

f) Then the appropriate antimicrobial discs were placed, distributed evenly on the inoculated plate.

g) Within 30 minutes of applying the antimicrobial discs, the plate was inverted and incubated aerobically at 35℃ for 16-18hrs.

h) After overnight incubation, the diameter of the zone of inhibition was measured in mm using a ruler on the underside of the plate.

i) Interpretation of zone size- using the interpretative charts, containing the zone sizes of each antimicrobials, as per CLSI guidelines, organisms were reported to be susceptible, intermediate or resistant to the particular antimicrobial drug.

j) Appropriate controls were used with each batch during this susceptibility testing.

   1. Escherichia coli- ATCC 25922
   2. Pseudomonas aeruginosa- ATCC 27853
   3. Staphylococcus aureus- ATCC 25923

PROCEDURE FOR DETECTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)-

Using the differential disk, Cefoxitin (30µg), Staphylococcus aureus isolates were categorized into methicillin sensitive and methicillin resistant strains.[52]

Cefoxitin(30µg)	Susceptible	Intermediate	Resistant
Zone size	>=22mm	-	< or =21mm

VANCOMYCIN SUSCEPTIBILITY TESTING AND MIC DETECTION PROCEDURE-

Vancomycin sensitivity was tested using Vancomycin screen agar (BHI agar with 6µg/ml of Vancomycin), where 10µl of bacterial suspension was spot inoculated onto this media and incubated overnight at 37℃ along with appropriate controls.[52]

After 24hrs of incubation, the sensitivity pattern was interpreted as follows-

1. If no visible growth at spot inoculated site- reported as sensitive to Vancomycin.
2. If visible growth (> 1 colony) at spot inoculated site was present –reported as resistant to Vancomycin.

E-TEST PROCEDURE-

Using an inoculating loop, 4-5 isolated colonies of Staphylococcus were transferred to a test tube containing peptone water and emulsified. Incubated it for 2-4hrs until the growth equal to a 0.5 McFarland turbidity standard was reached.
A sterile cotton swab was dipped into this inoculum suspension and pressed against the inside wall of the tube to remove excess fluid and then streaked over the entire surface of Mueller Hinton agar plate evenly in three directions. The surface of agar was allowed to dry completely and then an E-strip was applied to the agar surface with the MIC scale facing upwards. The plate was then incubated at 37℃ for overnight incubation. After 24 hrs of incubation, the MIC value was read at a point where the edge of inhibition ellipse intersects the strip.[52]

Vancomycin	Susceptible	Intermediate	Resistant
MIC(µg/ml)	< or =2	4-8	>=16

Among the Gram negative organisms identified, **ESBL producers** detected as follows-

Initial screening test done by disk diffusion method under CLSI guidelines using Cefotaxime (30µg) disk and Ceftazidime (30µg) disk which were applied on to Mueller Hinton agar plate inoculated with the test organism and incubated at 37℃ for 24hrs. Screening test denoted ESBL production if zone size was as follows- [52]

Cefotaxime (30µg)	< or =27mm
Ceftazidime (30µg)	< or =22mm

Phenotypic confirmatory test done by disk diffusion method under CLSI guidelines by combination disk test method using cefotaxime (30µg) disk and cefotaxime- clavulanic acid (30µg/10µg).

Combination disk test- Disks containing cephalosporin alone and in combination with clavulanic acid were applied onto Mueller Hinton agar plate inoculated with test organism and incubated at 37℃ for 24hrs.

Interpretation- Inhibition zone around cephalosporin disk combined with clavulanic acid is **>=5mm** larger than the inhibition zone around the disk with cephalosporin alone was considered **positive** for ESBL.

MOLECULAR METHODS-

Characterization of resistant bacterial isolates-

Polymerase chain reaction was performed to detect the resistant genes.

Materials required-

PureFast® Bacterial DNA minispin purification kit [Kit contains Lysozyme, Lysozyme digestion buffer, Proteinase-K, Binding buffer, Wash Buffer-1, Wash Buffer-2, Spin columns with collection tube and elution buffer.

Other requirements-

2X Red Dye PCR Master Mix, Agarose gel electrophoresis consumables and blaTEM, blaCTX-M, mecA Primers.

2X Master Mix:

It contains 2U of Taq DNA polymerase, 10X Taq reaction buffer, 2mM MgCl2, 1µl of 10mM dNTPs mix and RedDye PCR additives.

Agarose gel electrophoresis:

Agarose, 50X TAE buffer, 6X gel loading buffer and Ethidium bromide.

PCR:

blaTEM gene Primer mix - 5µl/reaction PCR Product: 260bp

blaCTX-M gene Primer mix - 5µl/reaction PCR Product: 295bp

mecA gene Primer mix - 5µl/reaction PCR Product: 220bp

EXTRACTION OF DNA-

PROTOCOL-

1. 1ml of overnight culture was centrifuged at 6000rpm for 5mins.
2. Supernatant discarded
3. Pellet was suspended in 0.2ml PBS.
4. 180µl of Lysozyme digestion buffer and 20µl of Lysozyme [10mg/ml] were added.
5. Incubated at 37^0C for 15mins.

6. 400µl of Binding buffer, 5µl of internal control template and 20µl of Proteinase K were added and mixed well by inverting several times.
7. Incubated at 56^0C for 15mins.
8. Then added 300µl of Ethanol and mixed well.
9. Transferred entire sample into the PureFast® spin column. Centrifuged for 1 min. discarded the flow-through and placed the column back into the same collection tube.
10. Added 500µl Wash buffer-1 to the PureFast® spin column. Centrifuged for 30-60 seconds and discarded the flow-through. The column was placed back into the same collection tube.
11. Then added 500µl Wash buffer-2 to the PureFast® spin column. Centrifuged for 30-60 seconds and discarded the flow-through. The column was placed back into the same collection tube.
12. Discarded the flow-through and centrifuged for an additional 1 min. This step was essential to avoid residual ethanol.
13. Transferred the PureFast® spin column into a fresh 1.5 ml micro-centrifuge tube.
14. Then added 100µl of Elution Buffer to the center of PureFast® spin column membrane.
15. Incubated for 1 min at room temperature and centrifuged for 2 mins.
16. Discarded the column and then stored the purified DNA at -20°C. Quality and Quantity of extracted DNA was checked by loading in 1% agarose gel and 5µl of extracted DNA was used for PCR amplification.

AMPLIFICATION OF DNA:

1. Reactions were set up as follows:

Components		Quantity
RedDye PCR Master Mix	-	10µl
Primer Mix	-	5µl
Purified Bacterial DNA	-	5µl
Total volume	-	20µl

2. Mixed gently and was spinned down briefly.

3. Placed into PCR machine and programmed it as follows:

Initial Denaturation	**:**	95℃ for 5 mins		
Denaturation	**:**	94℃ for 30secs]		
Annealing	**:**	58℃ for 30secs]	----	35 cycles
Extension	**:**	72℃for 30secs]		
Final extension	**:**	72℃ for 5 mins		

Loading:

a) Prepared 2% agarose gel. [2gm of agarose in 100ml of 1X TAE buffer]

b) Electrophoresis was run at 50V till the dye reached three fourth distances and observed the bands in **UV Transilluminator.**

Agarose gel electrophoresis:

1. Prepared 2% agarose. (2gm agarose in 100ml of 1X TAE buffer and melted using micro oven)
2. When the agarose gel temperature was around 60℃, added 5µl of Ethidium bromide.
3. Poured warm agarose solution slowly into the gel platform.
4. Kept the gel set undisturbed till the agarose solidified.
5. Poured 1XTAE buffer into submarine gel tank.
6. Carefully the gel platform was placed into the electrophoretic tank which was filled with buffer at a level of 0.5cm above the gel.
7. PCR Samples were loaded after mixed with gel loading dye along with 10µl of 100bp DNA Ladder. [100bp, 200bp, 300bp, 400bp, 500bp, 600bp, 700bp, 800bp, 900bp, 1000bp and 1500bp]
8. Electrophoresis was run at 50V till the dye reached three fourth distance of the gel.
9. The gel was observed under the UV Transilluminator and observed the bands pattern which detected the presence of DNA.

Determination of Inflammatory markers:

About 2ml blood sample was collected under sterile aseptic precautions and allowed to clot in a dry test tube, serum separated and used for the estimation of inflammatory markers.

1. C-Reactive protein estimation-

CRP estimation was done using PROTON-CRP ESTIMATION KIT-GenX CRP slide latex agglutination test kit.

Principle-

The test is based on the principle of latex agglutination. CRP estimation was done using uniform latex particles coated with anti-human CRP.When latex reagent was mixed with the test serum containing CRP, at a level greater than 0.6mg/dl, it forms visible agglutination. If the concentration of CRP lesser than 0.6mg/dl, then no agglutination.

Materials required-

1. Anti-CRP latex reagent
2. Positive control
3. Negative control
4. Others- slides, droppers, mixing sticks.

Sample- serum

It involves-qualitative test and semi-quantitative test.

i) Qualitative test-

Procedure-

1. One drop (approx.40-50µl) of test serum sample, positive control and negative control was placed in separate circles of the glass slide by using the sample dropper.
2. Then one drop of latex reagent was added in each of these circles with the dropper.
3. The contents of each circle were mixed separately and were spread in the entire circle with the mixing sticks.
4. Then the slide was rocked gently for 2 minutes and looked for any visible agglutination.
5. The results should not be read after 2 minutes.

Interpretation-

Marked agglutination- CRP positive (>0.6mg/dl)

No agglutination- CRP negative (<0.6mg/dl)

ii) Semi- quantitative determination-

Procedure-

1. The sera found positive were retested by preparing serial dilutions of the test sample (1:2, 1:4, 1:8, 1:16, and so on) using 0.9% sodium chloride solution in clean labelled test tubes as below:

Tube no.	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**Saline control**
Serum dilution	1:2	1:4	1:8	1:16	1:32	1:64	-
Saline	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
Patient's serum	0.5ml	-	-	-	-	-	-
Transfer diluted serum from previous tube	-	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	-

Each dilution was tested like the qualitative procedure until no further agglutination was observed.

CRP concentration was estimated for the highest dilution at which visible agglutination occurred and calculated by using the formula-

CRP (mg/l) - highest dilution with positive reaction X reagent sensitivity (6mg/l) The titres for the various dilutions are as follows-

DILUTION	TITRE
1:2	12mg/l
1:4	24mg/l
1:8	48mg/l
1:16	96mg/l
1:32	192mg/l
1:64	384mg/l

Estimation of Interleukin-6-

IL-6 was done using Human Interleukin-6 Kit, Krishgen Biosystems

Principle-

The test is based on the principle of sandwich ELISA method which quantifies the target cytokine between two layers of antibodies (i.e. capture and detection antibody). The cytokine to be measured contains at least two antigenic epitope capable of binding to antibody, since at least two antibodies act in the sandwich. The advantage of Sandwich ELISA is that the sample does not have to be purified before analysis, and the assay can be very sensitive up to pg/ml levels (more sensitive than direct or indirect ELISA).

Materials required-

1. Microtiter Coated Plate (12X8 wells)
2. Recombinant Human IL-6 Standard
3. Human IL-6 Biotin Conjugated Detection Antibody
4. Concentrated Avidin Horseradish Peroxidase
5. Wash Buffer (20X) – 25ml
6. . Assay Diluent (5X) - 10ml
7. Assay Diluent – 6ml (ready to use)
8. TMB Substrate – 12ml
9. Stop Solution – 12ml

Sample-serum

REAGENT PREPARATION-

1. WASH BUFFER (20X):

   To make wash buffer (1X), 5ml of wash buffer (20X) added to 95ml of distilled water- working solution.

2. ASSAY DILUENT (1X):

   To make assay diluent (1X), 10ml of assay diluent (5X) added to 40ml of distilled water- working solution.

3. STANDARD (RECOMBINANT HUMAN-IL-6 LYOPHILIZED, 200pg/ml):

   Lyophilized Human IL-6 standard reconstituted with 650µl of distilled water to achieve final concentration of 200pg/ml- used as the top standard to perform serial dilutions.

4. BIOTIN CONJUGATED DETECTION ANTIBODY (50µl):

   20µl of detection antibody solution added to 4980µl of assay diluents (1X) to make the final volume to 5ml.

5. CONCENTRATED AVIDIN-HRP (50µl):

   20µl of Avidin-HRP added to 4980µl of assay diluents (1X) to make the final volume to 5ml.

ASSAY PROCEDURE-

1. All reagents were brought to room temperature prior to use.
2. Added 100µl/well of assay diluent (ready to use preparation) only along with standards which were used for serial dilutions.
3. Then added 100µl/well of Standards and Samples to the plate, where only standards were duplicated and performed two-fold serial dilutions of the 200pg/ml top standard, within the plate. Thus, the Human IL-6 standard concentrations were 200pg/ml, 100pg/ml, 50pg/ml, 25pg/ml, 12.5pg/ml, 6.25pg/ml and 3.13pg/ml. Assay Diluent (1X) served as the zero standard (0 pg/ml). Sealed the plate and incubated for 2 hours at room Temperature (18-25°C).
4. Aspirated and washed the plate 4 times with Wash Buffer (1X) and blotted the residual buffer by firmly tapping plate upside down on absorbent paper. Wiped of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes were performed similarly.

5. Then added 100µl of diluted Detection Antibody solution to each well sealed the plate and incubated for 1 hour at room Temperature (18-25°C).
6. Washed the plate 4 times with Wash Buffer (1X) as in step 4.
7. Added 100µl of diluted Avidin-HRP solution to each well sealed the plate and incubated for 30 minutes at room Temperature (18-25°C).
8. Washed the plate 4 times with Wash Buffer (1X) as in step 4.
9. Added 100µl of TMB Substrate solution and incubated in the dark for 30 minutes. Positive wells turned bluish in color. No need to seal the plate during this step.
10. The reaction was stopped by adding 100µl of Stop Solution to each well. Positive wells turned from blue to yellow.
11. Absorbance was read at 450 nm within 30 minutes of stopping the reaction.

CALCULATION OF RESULT:

1. Using a semi-log graph paper, the optical densities of each standard were plotted on Y-axis and the corresponding concentration of the standards on the X-axis.
2. The best fit straight line was drawn through the standard points.
3. To determine the unknown cytokine concentration, a horizontal line was drawn corresponding to the optical density obtained for each sample plotted on Y- axis, intersecting the standard curve.
4. At the point of intersection, a vertical line was drawn to the X-axis and read the cytokine concentration which was expressed in pg/ml.

STATISTICAL ANALYSIS-

- Statistical analysis for the collected data was done using SPSS software 21.
- The demographic variables were expressed in frequency and percentage.
- For Quantitative variables, mean and standard deviations were determined.
- Validity of the tests were determined by calculating the sensitivity, specificity, positive predictive value, negative predictive value, 95% confidence intervals.

Results

RESULTS

The study was done during a period of 1year from March 2017- February 2018 at Institute of Microbiology in collaboration with the Institute of Internal Medicine and Surgery. The study group included 150 patients in the age group > 18yrs with clinical suspicion of sepsis admitted within 48hrs in Medical, surgical wards and Intensive Care Units at Rajiv Gandhi Government General Hospital.

TABLE-1

AGE DISTRIBUTION OF PATIENTS WITH SUSPECTED SEPSIS (n=150)

AGE (yrs)	NO. OF PATIENTS	PERCENT
19-29	31	20.7
30-39	29	19.3
40-49	41	27.3
50-59	26	17.3
60 & above	23	15.3
TOTAL	150	100

CHART 1

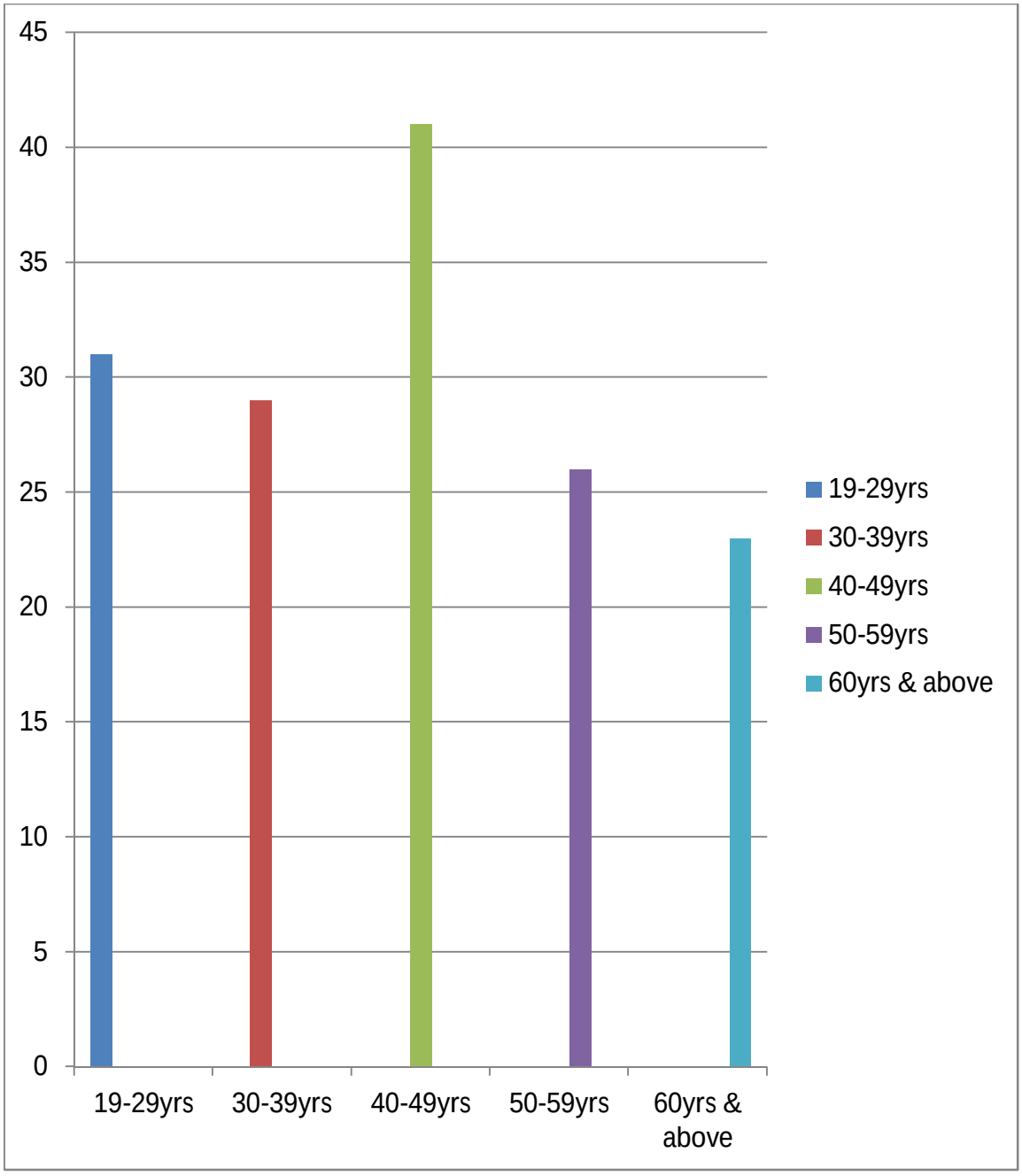

45
40
35
30
25
20
15
10
5
0
19-29yrs
30-39yrs
40-49yrs
50-59yrs
60yrs & above
19-29yrs
30-39yrs
40-49yrs
50-59yrs
60yrs & above

TABLE-2

SEX DISTRIBUTION OF PATIENTS WITH SUSPECTED SEPSIS (n=150)

SEX	NO. OF PATIENTS	PERCENT
MALE	91	60.7
FEMALE	59	39.3
TOTAL	150	100

CHART -2

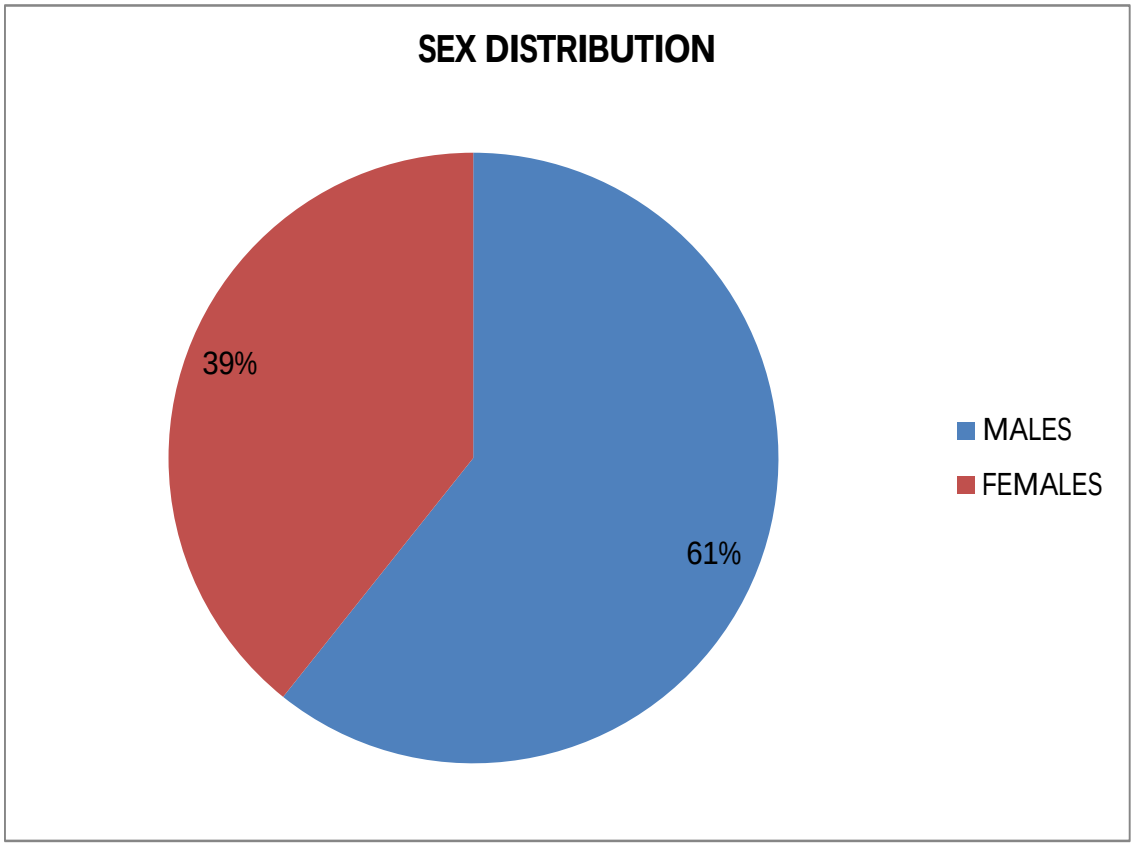
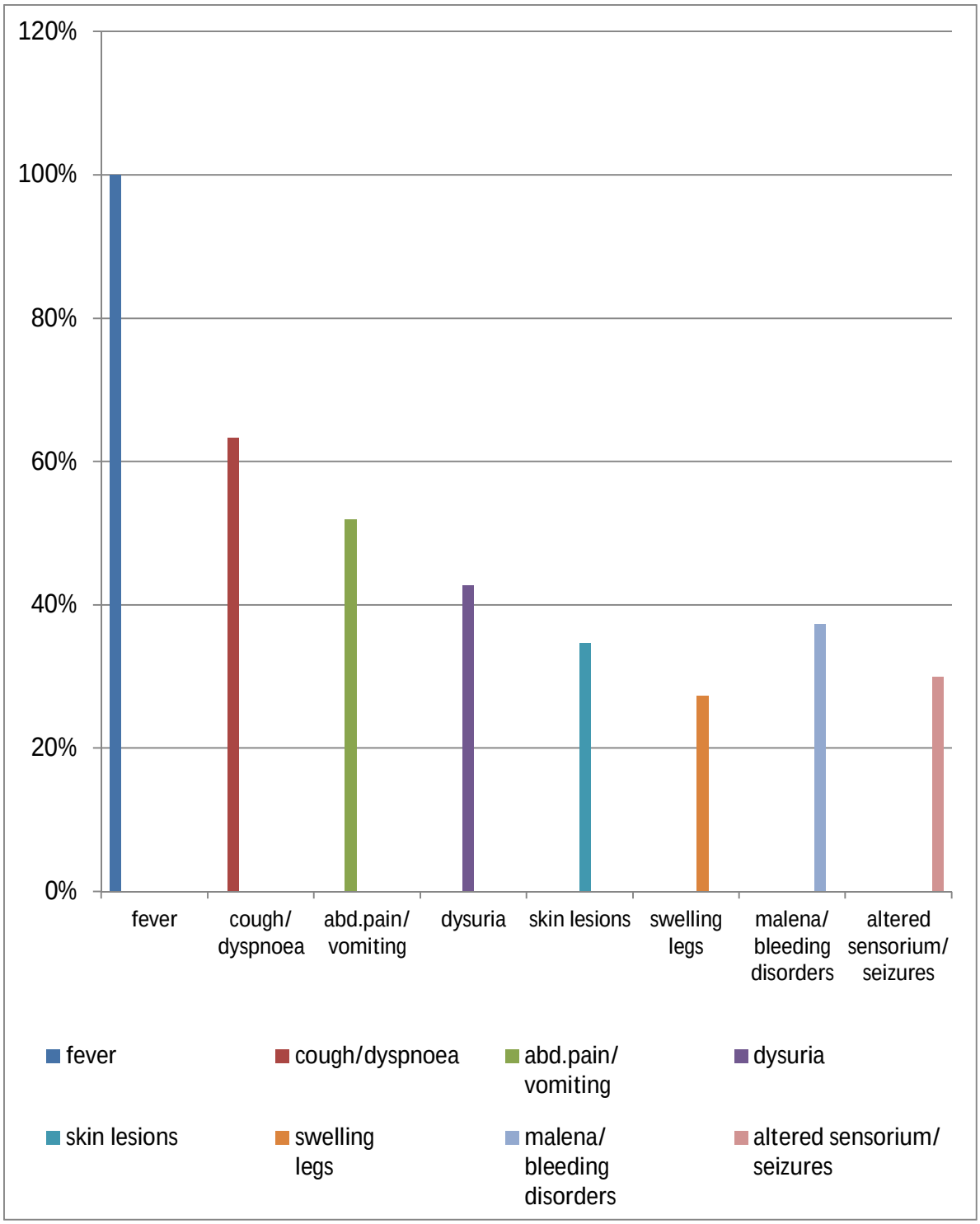


CHART 3

CLINICAL PROFILE OF PATIENTS WITH SUSPECTED SEPSIS (n=150)



RESULTS OF BLOOD CULTURE-

Blood culture was done in 150 patients with clinical suspicion of sepsis, out of which community acquired bloodstream infection was detected in 12 patients (8%)

CHART 4

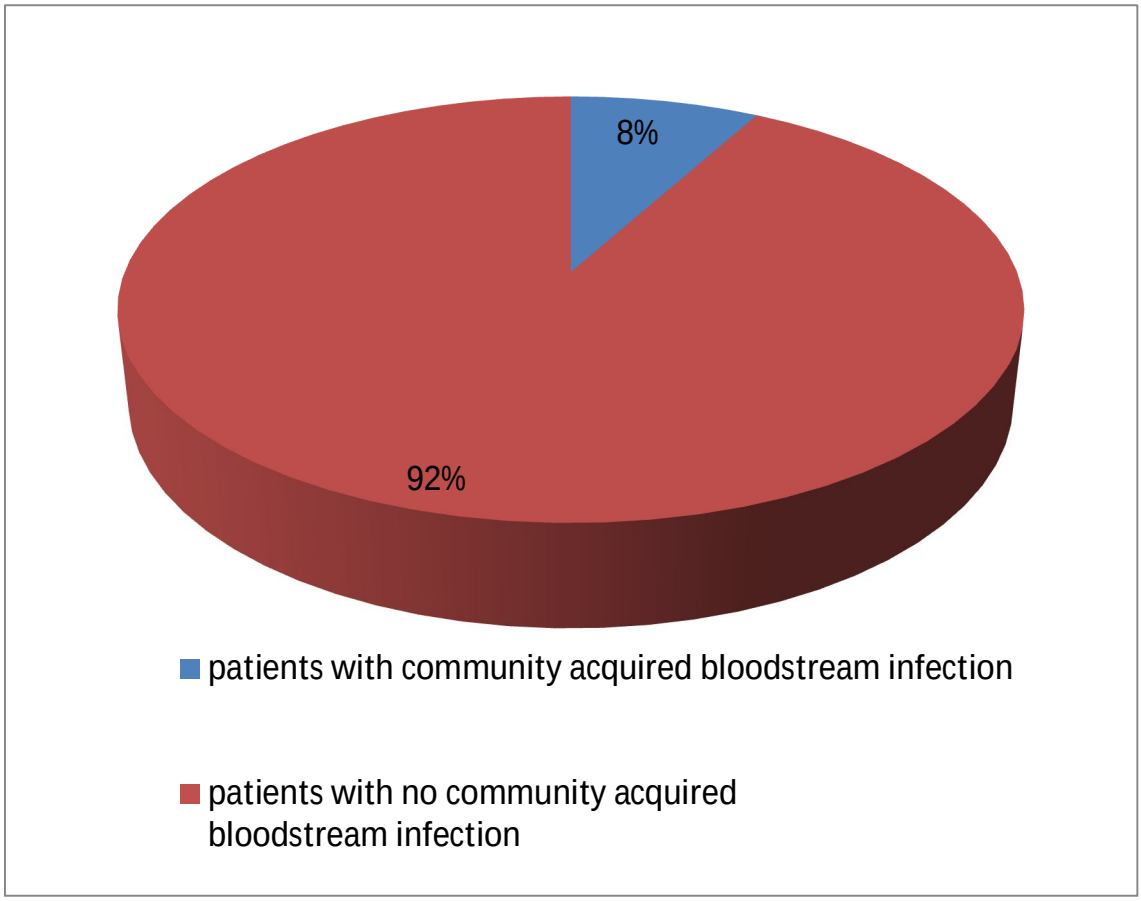


TABLE-3

AGE DISTRIBUTION OF PATIENTS WITH COMMUNITY ACQUIRED BLOODSTREAM INFECTION

AGE (yrs)	NO. OF PATIENTS WITH COMMUNITY ACQUIRED BSI	PERCENT
51-60	6	50
61-70	4	33.3
71-80	1	8.3
>80	1	8.3
Total	12	100

Above table shows, majority (50%) falls under the age group 51-60yrs.

CHART 5

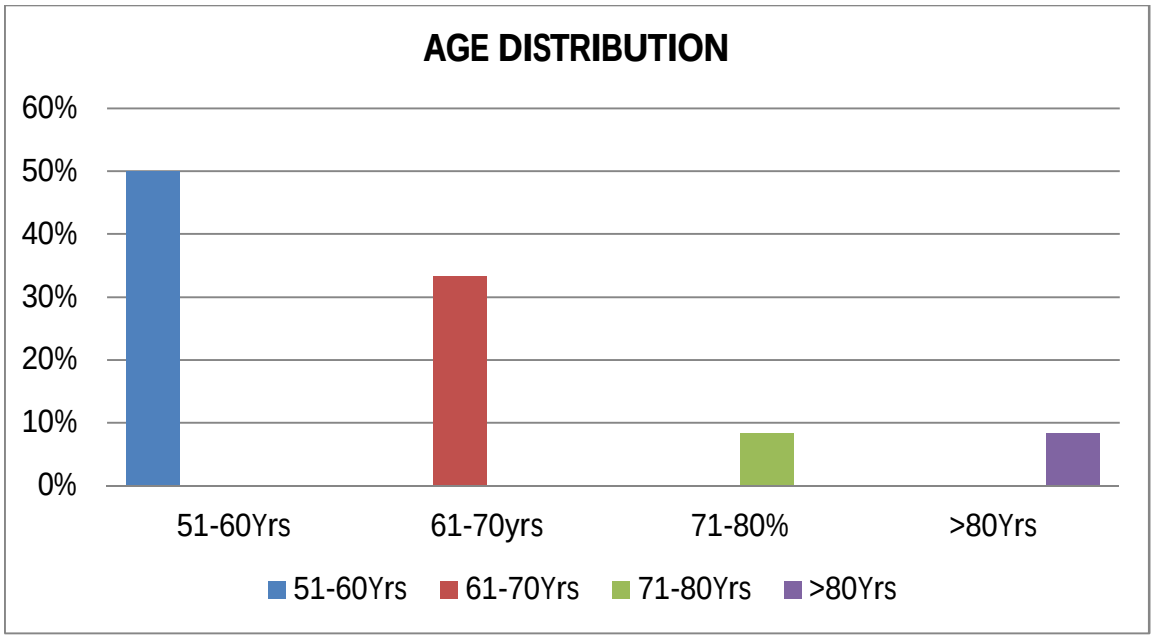


TABLE-4

SEX DISTRIBUTION OF PATIENTS WITH COMMUNITY ACQUIRED BLOODSTREAM INFECTION

SEX	NO. OF PATIENTS	PERCENT
MALE	9	75
FEMALE	3	25
TOTAL	12	100

CHART 6

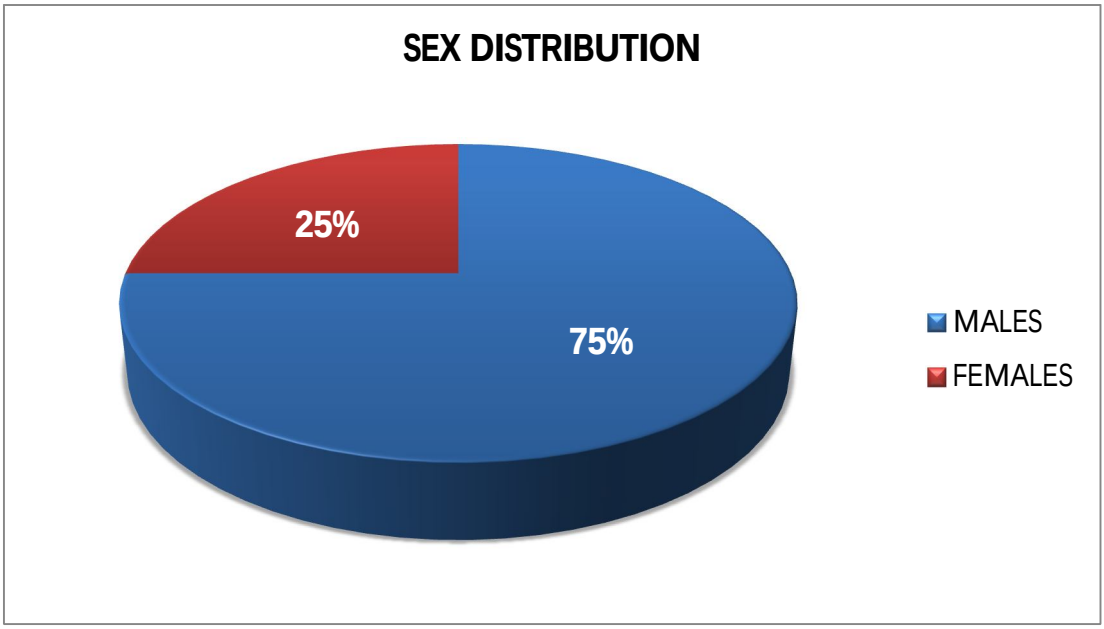


Table 4 reveals that males were predominantly affected in patients with community acquired BSI

CHART 7

ORGANISMS ISOLATED BY BLOOD CULTURE IN COMMUNITY ACQUIRED BLOODSTREAM INFECTION

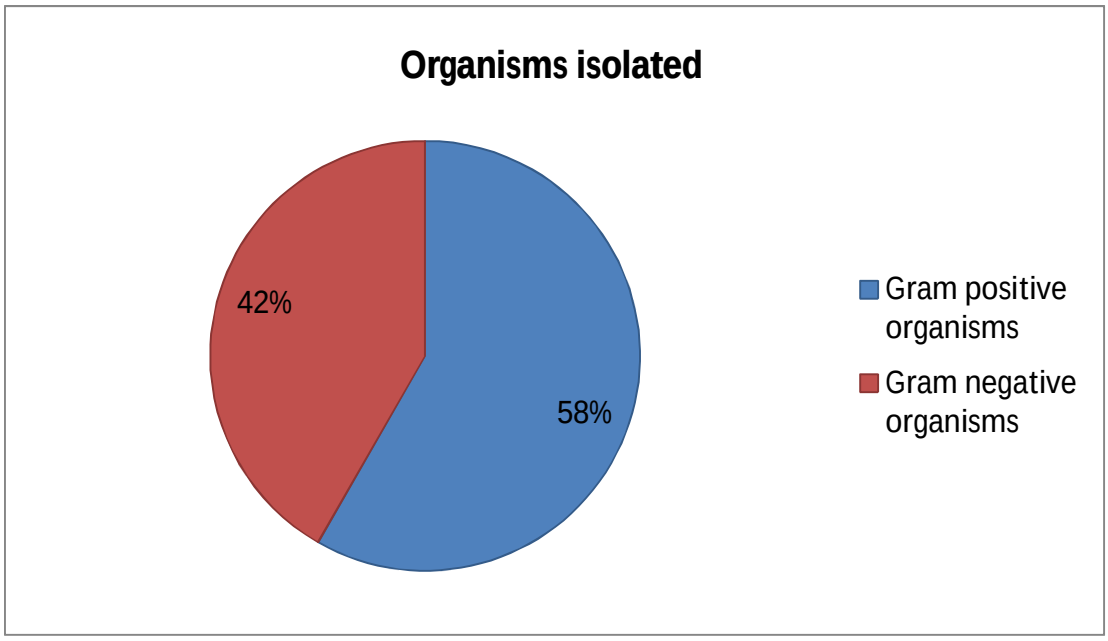


CHART 8

WARD WISE DISTRIBUTION OF GRAM POSITIVE ISOLATES OBTAINED

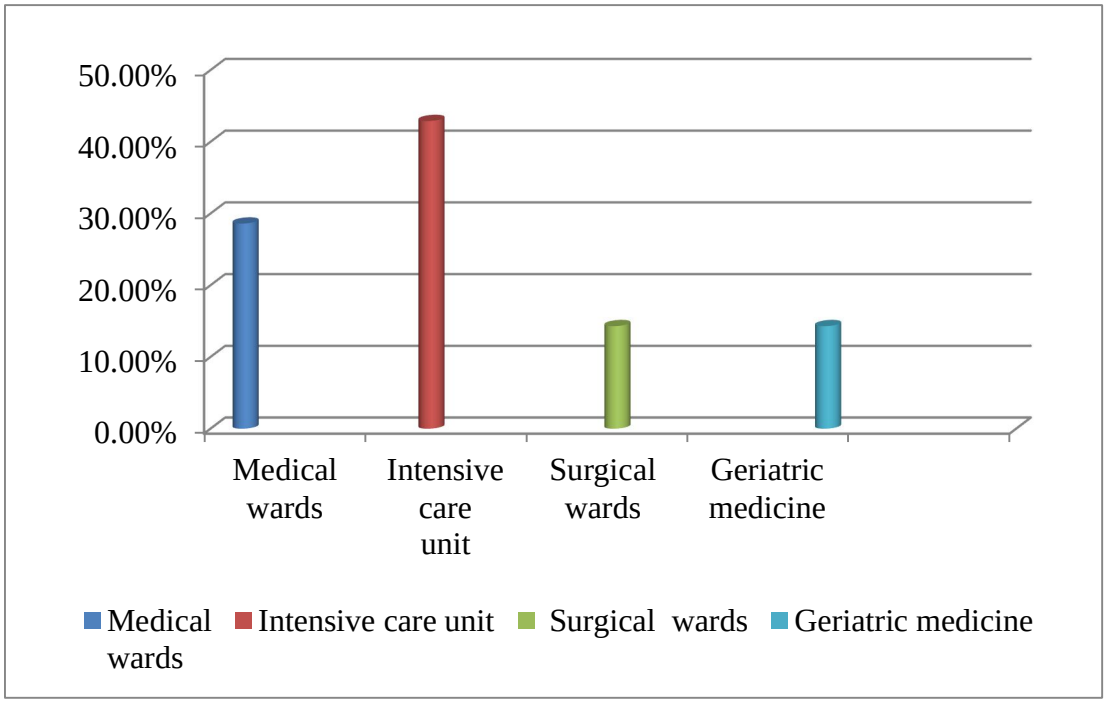


CHART 9

WARD WISE DISTRIBUTION OF GRAM NEGATIVE ISOLATES OBTAINED

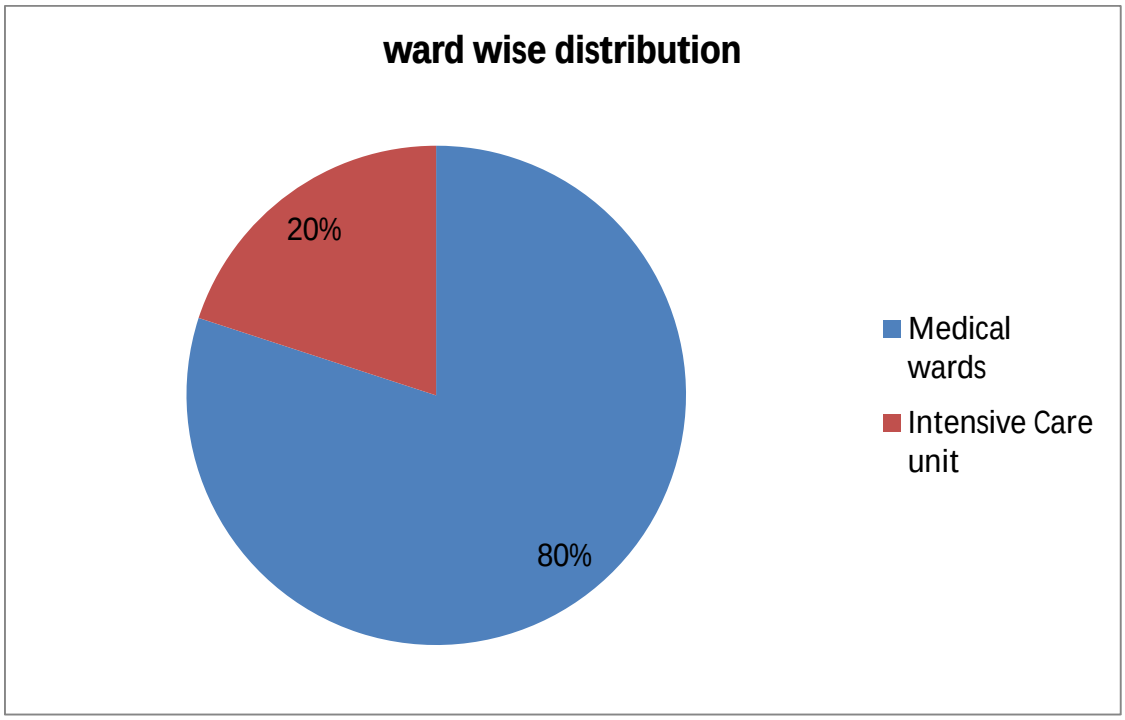


TABLE-5

GRAM POSITIVE ORGANISMS

ORGANISMS	NO. ISOLATED	PERCENT
Staphylococcus aureus (MSSA)	6	85.7
Staphylococcus aureus(MRSA)	1	14.3
Total	7	100

TABLE-6

CRP LEVELS AMONG GRAM POSITIVE ORGANISMS

ORGANISMS	NO. ISOLATED	CRP RANGE
Staphylococcus aureus (MSSA)	6	190-200mg/l
Staphylococcus aureus (MRSA)	1	

TABLE-7

IL-6 LEVELS AMONG GRAM POSITIVE ORGANISMS

ORGANISMS	NO. ISOLATED	IL-6 RANGE
Staphylococcus aureus (MSSA)	6	300-900 pg/ml
Staphylococcus aureus (MRSA)	1	

Tables-5,6&7 depicts that among Gram positive organisms detected, 85.7% were MSSA and 14.3% were MRSA isolates with CRP conc. ranged between 190-200mg/l and IL-6 conc. ranged between 300-900pg/ml.

TABLE-8

GRAM NEGATIVE ORGANISMS

ORGANISMS	NO. ISOLATED	PERCENT
E.coli	3	60
Pseudomonas aeruginosa	1	20
Acinetobacter baumannii	1	20
Total	5	100

Above table shows among the Gram negative organisms isolated, E.coli contributes 60%, Pseudomonas aeruginosa and Acinetobacter baumannii contributes each 20% respectively.

TABLE-9

CRP LEVELS AMONG GRAM NEGATIVE ORGANISMS

ORGANISMS	NO. ISOLATED	CRP RANGE
E.coli	3	190-400 mg/l
Pseudomonas aeruginosa	1	
Acinetobacter spp.	1	

TABLE-10

IL-6 LEVELS AMONG GRAM NEGATIVE ORGANISMS

ORGANISMS	NO. ISOLATED	IL-6 RANGE
E.coli	3	900-1200 pg/ml
Pseudomonas aeruginosa	1	1000-1200 pg/ml
Acinetobacter baumannii	1	600-800 pg/ml

TABLE-11

RESISTANT STRAINS AMONG GRAM NEGATIVE ORGANISMS

CHARACTERS	VALUE
Total Gram negative bacilli	5
Resistant strains (ESBL producers)	3
Percentage of resistant strains among Gram negative bacilli	60%

From above table, Among the Gram negative organisms isolated, 60% were ESBL producers.

TABLE-12

CRP & IL-6 LEVELS AMONG RESISTANT STRAINS

RESISTANT STRAINS	CRP LEVEL	RESISTANT STRAINS	IL-6 LEVEL
MRSA(1)	192 mg/l	MRSA(1)	826pg/ml
ESBL(3)	300-400mg/l	ESBL(3)	900-1200pg/ml

TABLE-13

ANTIMICROBIAL SUSCEPTIBILITY PATTERN AMONG GRAM POSITIVE ORGANISMS

ORGANISM	METHICILLIN SENSITIVE STAPHYLOCOCCUS AUREUS (MSSA)		METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)	
NUMBER ISOLATED	6		1	
DRUGS	S (%)	R (%)	S (%)	R (%)
Ciprofloxacin	50	50	100	0
Penicillin	100	0	0	100
Cotrimoxazole	33.3	66.7	0	100
Erythromycin	100	0	100	0
Linezolid	100	0	100	0
Tetracycline	100	0	0	100
Vancomycin	100	0	100	0

TABLE-14

ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE ORGANISMS

ORGANISM	ESCHERICHIA COLI		PSEUDOMONAS AERUGINOSA		ACINETOBACTER BAUMANNII	
NO. ISOLATED	3		1		1	
DRUGS	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Amikacin	100	0	100	0	0	100
Gentamicin	33.3	66.7	100	0	0	100
Ciprofloxacin	0	100	100	0	0	100
Cotrimoxazole	0	100	-		0	100
Ampicillin	0	100	-		-	
Cefotaxime	0	100	-		-	
Cefotaxime-clavulanic acid	100	0	-		-	
Ceftazidime	0	100	0	100	0	100
Tetracycline	100	0			100	0
Piperacillin-Tazobactum			100	0	100	0
Imipenem	-		100	0	100	0

TABLE-15

CO-MORBIDITIES AND RISK FACTORS ASSOCIATED WITH BSI IN THIS STUDY

CO-MORBIDITIES AND RISK FACTORS	NO. OF PATIENTS	PERCENT
DIABETES	9	75
HYPERTENSION	4	33.3
SMOKING	3	25
ALCOHOLISM	4	33.3
ANAEMIA	7	58.3

CHART 10

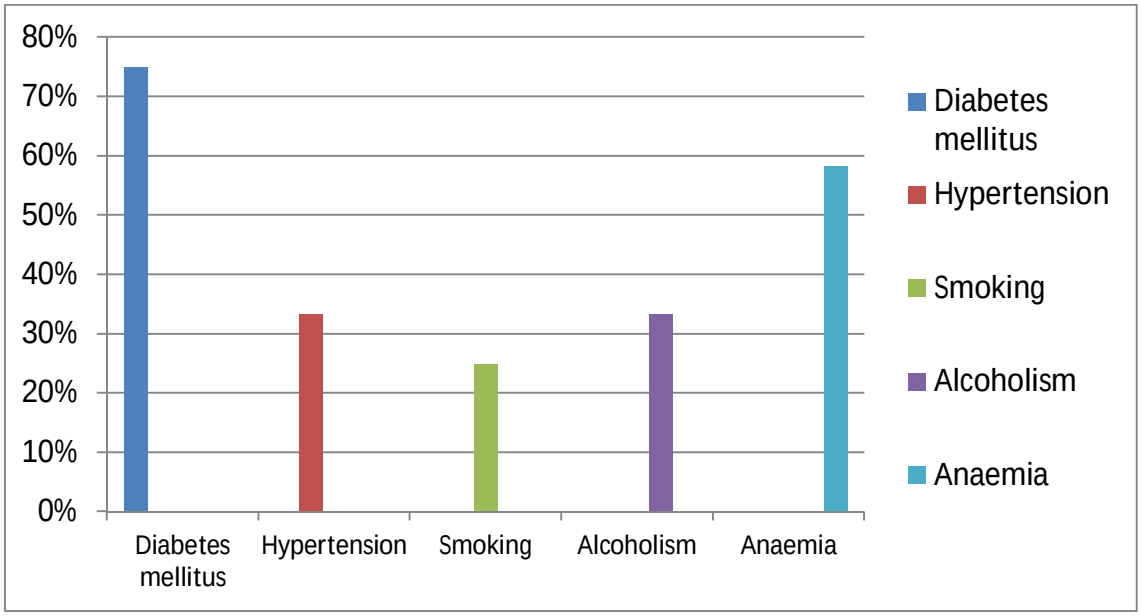


Table 15 shows that diabetes mellitus (75%) and anaemia (58.3%) were the predominant risk factors.

TABLE-16

PRIMARY SYSTEM INVOVEMENT IN COMMUNITY ACQUIRED BSI PATIENTS

SYSTEM INVOVLED	NO. OF PATIENTS	PERCENT
RESPIRATORY	8	66.7
INTRAABDOMINAL	2	16.7
RENAL	1	8.3
SKIN AND SOFT TISSUES	1	8.3
TOTAL	12	100

Respiratory system was the predominant primary system involved (66.7%) in patients with community acquired BSI.

TABLE-17

HEMATOLOGICAL PARAMETERS IN BLOOD CULTURE NEGATIVE CASES

	N	Minimum	Maximum	Mean	Std. Deviation
Hemoglobin	138	8.5	16.4	11.888	1.3862
Total count	138	3400.0	18200.0	13134.058	2606.9110
ESR	138	10.0	28.0	17.717	3.8341
MCV	138	71.4	92.9	82.915	4.4553
MCH	138	23.6	34.6	27.693	2.1381
MCHC	138	29.6	38.5	32.950	1.4017

TABLE-18

HEMATOLOGICAL PARAMETERS IN BLOOD CULTURE POSITIVE CASES

	N	Minimum	Maximum	Mean	Std. Deviation
Hemoglobin	12	8.4	13.0	10.150	1.4619
Total count	12	18600.0	23800.0	21600.000	1616.3933
ESR	12	22.0	28.0	24.750	1.7645
MCV	12	74.2	102.2	80.408	7.8102
MCH	12	23.3	42.6	28.192	5.6250
MCHC	12	29.8	36.2	31.750	1.8103

TABLE-19 : PLATELET COUNT

PLATELET COUNT	FREQUENCY	PERCENT
NORMAL	124	82.7
ABNORMAL	26	17.3
TOTAL	150	100

Table 17, 18, 19 shows correlation statistically significant with a p value 0.05.

TABLE-20

C-reactive protein (CRP) & Blood culture positivity

CRP conc. range	**6-10 mg/l**	**11-70mg/l**	**90-190mg/l**	**190-400 mg/l**
CRP	32 (21.3%)	90 (60%)	16 (10.7%)	12 (8%)
BLOOD CULTURE POSITIVE	-	-	-	12

TABLE-21

IL-6 & BLOOD CULTURE POSITIVITY

IL-6 conc. range	**3-11 pg/ml**	**12-60pg/ml**	**70-120pg/ml**	**200pg/ml &above**
IL-6	52 (34.7%)	28 (28.6%)	58 (59.2%)	12 (12.2%)
BLOOD CULTURE POSITIVE	-	-	-	12

Table 20, 21- depicts that both CRP and IL-6 levels were found elevated in blood culture positive patients (correlation significant with a p value 0.01).

TABLE-22

OUTCOME OF PATIENTS WITH COMMUNITY ACQUIRED BSI

OUTCOME	FREQUENCY	PERCENT
RECOVERED	9	75
EXPIRED	3	25
TOTAL	12	100

Among 12 patients with community acquired BSI, 9 patients (75%) got recovered and 3 patients (25%) expired.

CHART 11

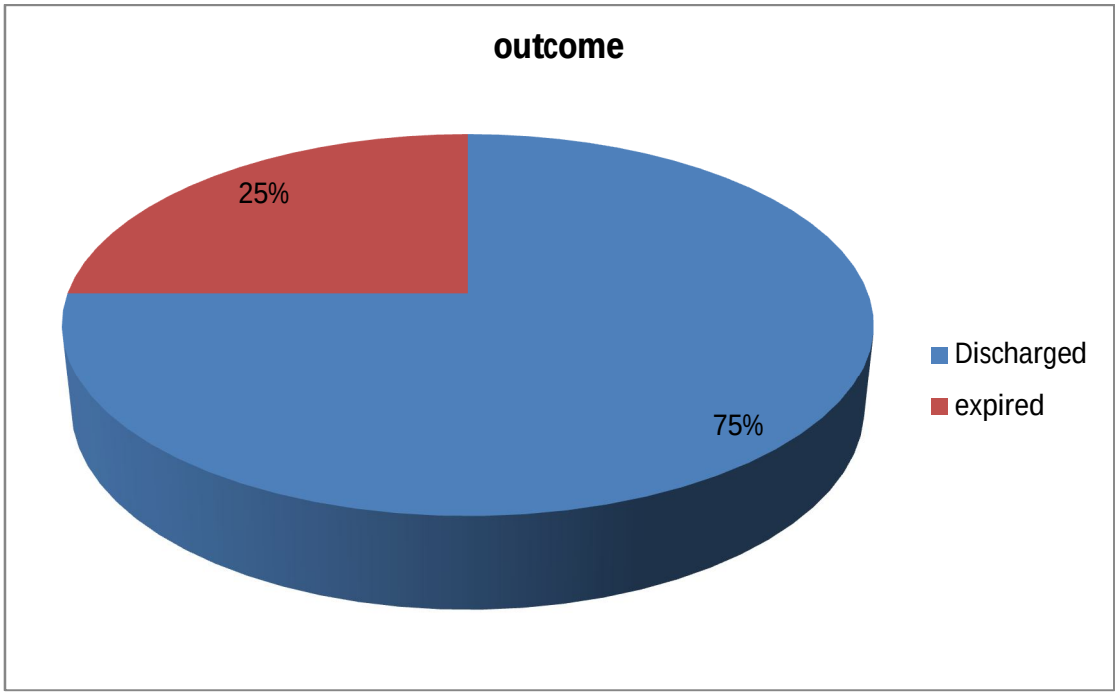


TABLE-23

CRP & IL-6 LEVELS AND OUTCOME OF PATIENTS WITH COMMUNITY ACQUIRED BSI

CRP RANGE	IL-6 RANGE	OUTCOME
300-400mg/l	900-1200pg/ml	Expired(3)
190-200mg/l	400-800pg/ml	recovered

TABLE-24 OUTCOME IN CULTURE NEGATIVE CASES

SIRS CRITERIA FULFILLED BUT CULTURE NEGATIVE	CRP RANGE	IL-6 RANGE	OUTCOME
136(98.6%)	6-100mg/l	3-96 pg/ml	recovered
2(1.4%)	90-200mg/l	80-120pg/ml	expired

TABLE-25 CRP POSITIVITY

CRP	FREQUENCY	PERCENT
POSITIVE	118	78.7
NEGATIVE	32	21.3
TOTAL	150	100

TABLE-26 IL-6 POSITIVITY

IL-6	FREQUENCY	PERCENT
POSITIVE	103	68.7
NEGATIVE	47	31.3
TOTAL	150	100

TABLE-27

COMPARISON OF IL-6 WITH CRP AS BETTER EARLY SEPSIS MARKER

IL-6 Vs CRP	ESTIMATED VALUE	95%CONFIDENCE INTERVAL
SENSITIVITY	83%	75%-89%
SPECIFICITY	84%	66%-94%
POSITIVE PREDICTIVE VALUE	95%	88%-98%
NEGATIVE PREDICTIVE VALUE	57%	42%-71%

Above table27 shows that IL-6 was both sensitive and specific early biomarker of sepsis than CRP in this study.

TABLE-28

AREA UNDER THE CURVE FOR IL-6 AND CRP

TEST VARIABLES	AREA UNDER THE CURVE
IL-6	67%
CRP	61%

CHART 12

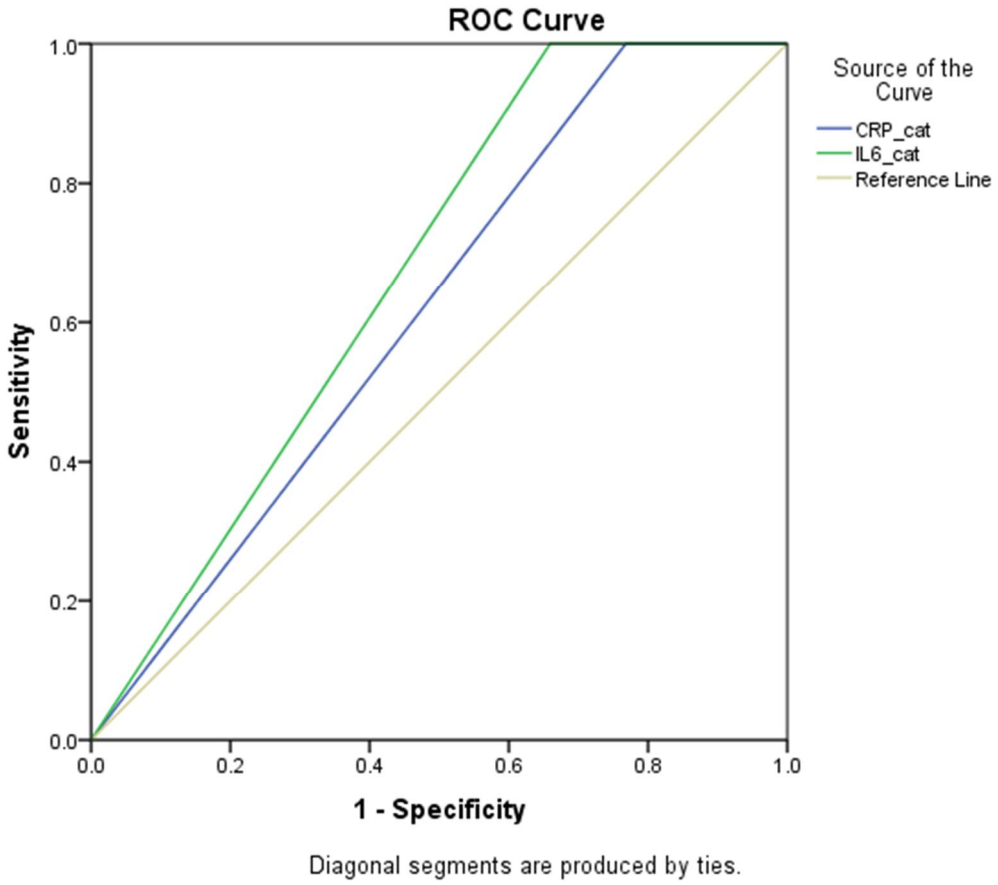


Table 28 shows that area under the curve for IL-6 was more than the CRP.

TABLE-29

MORTALITY PREDICTION IN PATIENTS WITH COMMUNITY ACQUIRED SEPSIS WITH IL-6 CONCENTRATION-

TEST VARIABLE	MEAN ESTIMATED VALUE	MEDIAN ESTIMATED VALUE
IL-6 (pg/ml)	1040	1180

CHART 13

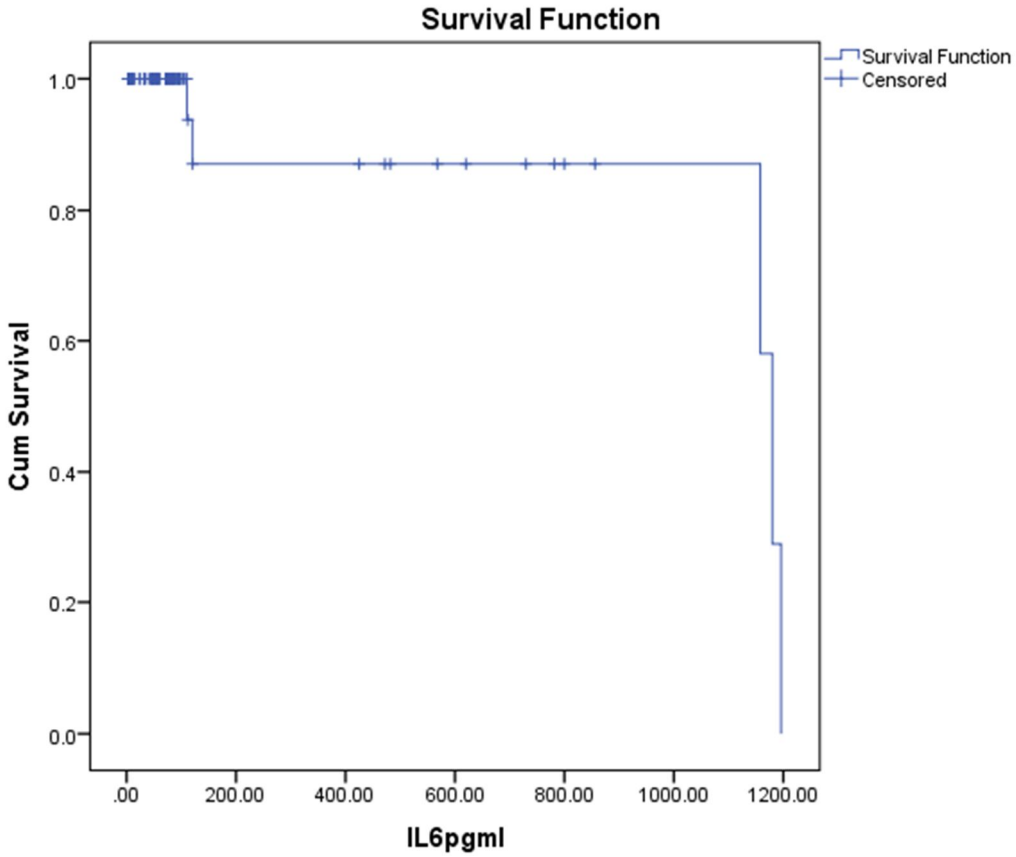


TABLE-30

MORTALITY PREDICTION IN PATIENTS WITH COMMUNITY ACQUIRED SEPSIS WITH CRP CONCENTRATION

TEST VARIABLE	MEAN ESTIMATED VALUE	MEDIAN ESTIMATED VALUE
CRP(mg/l)	350	384

CHART 14

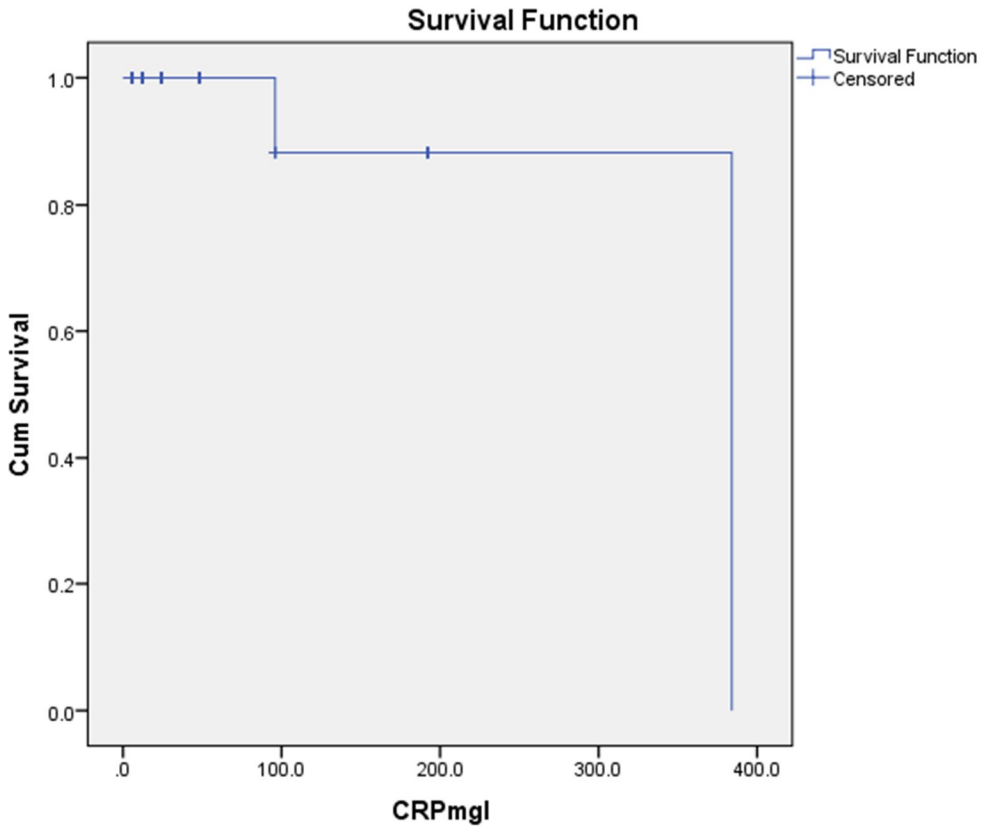


Table 29, 30 depicts that the mortality in patients with community acquired BSI could be predicted with median estimated value of IL-6 conc. of 1180pg/ml and CRP conc. of 384mg/l statistically.

TABLE-31

MOLECULAR IDENTIFICATION OF ANTIMICROBIAL RESISTANT GENES- BY PCR

RESISTANT STRAINS	PRIMERS	RESULT
ESBL PRODUCERS(3)	blaTEM	POSITIVE
ESBL PRODUCERS(3)	blaCTX-M	POSITIVE
MRSA STRAIN(1)	mecA	POSITIVE

Discussion

DISCUSSION

Bloodstream infections are an important cause of mortality and also morbidity related to sepsis. This study was focussed to know the burden of community acquired bloodstream infections in our settings and also to find a better diagnostic marker in detection of infections and sepsis.

During the study period of 1 year from March 2017- February 2018, blood culture was done in 150 patients with clinical suspicion of sepsis within 48hrs of hospital admission. Out of which, community acquired bloodstream infection was detected in 12 patients (8%), in this study. Tufail Soomro et al[53] (2016) concluded in their study that the frequency and incidence of community acquired bloodstream infection was 7.6%. B Sigauque et al [54] in their study had identified community acquired bloodstream infection in 8% of patients on hospital admission correlating well with our study. In a cohort study of 3901 patients with community acquired sepsis conducted by Nathan I. Shapiro et al, [55] the incidence of bloodstream infection at hospital admission was 8.2%.

In the present study, patients in the age group between 51-60yrs were predominantly affected with community acquired bloodstream infection (50%) and males were the predominantly affected groups (75%) than females (25%). In the study conducted by J. Goncalves- Pereira et al[56] and in the study of Ssali FN et al [57] males were predominantly affected with community acquired bloodstream

infection. In Tufail Soomro et al[53] study, females were the predominantly affected groups (61.5%).

In the present study, out of 12 patients with community acquired bloodstream infection, the frequency and distribution of pathogens were 58% Gram positive organisms and 42% Gram negative organisms.

Among the Gram positive organisms, 85.7% were methicillin sensitive Staphylococcus aureus (MSSA) and 14.3% were methicillin resistant Staphylococcus aureus (MRSA). Hence, among the Gram positive organisms, 14.3% were found to be resistant pathogens. In the study conducted by J. Goncalves- Pereira et al[56] also the predominant Gram positive organism isolated were methicillin sensitive Staphylococcus aureus and the predominant Gram negative organisms identified were Escherichia coli. In a study done by Klevens R M et al, [58] incidence of community associated methicillin resistant Staphylococcus aureus infection was found to be 14%.

In this study, among the Gram negative organisms, Escherichia coli contributed 60%, Pseudomonas aeruginosa and Acinetobacter baumannii each contributed 20% respectively. The study by Parkins MD et al, [59] also showed that the incidence of community acquired bloodstream infection cases caused by Pseudomonas aeruginosa were 21%, well correlates with our study. Also, in a study of Chung-Ting Chen et al[60] (2017), Acinetobacter baumannii isolates were identified as the causatives of community acquired bloodstream infections and for these isolates, respiratory tract was the primary source involved which matches

with the present study where the Acinetobacter baumannii isolate identified was acquired from respiratory tract as primary source of infection. Among the Gram negative organisms isolated, 60% were found to be resistant pathogens especially, extended spectrum beta-lactamase (ESBL) producers among Escherichia coli organisms. Quan J et al, [61] (2017) study revealed 56% of ESBL producing E.coli isolates were identified in community acquired bloodstream infections.

In the present study, among Gram positive organisms isolated, methicillin sensitive Staphylococcus aureus were highly sensitive to Penicillin(100%), Erythromycin(100%), Tetracycline(100%), Linezolid(100%), Vancomycin(100%) and were resistant to Cotrimoxazole(66.7%) and Ciprofloxacin(50%).Methicillin resistant Staphylococcus aureus was highly sensitive to Ciprofloxacin(100%), Erythromycin(100%), Linezolid(100%), Vancomycin(100%) and were highly resistant to Penicillin(100%), Cotrimoxazole(100%) and Tetracycline(100%).

Among the Gram negative organisms isolated, Escherichia coli isolates were highly sensitive to Amikacin(100%), Tetracycline(100%) and were found highly resistant to Ciprofloxacin, Cotrimoxazole, Ampicillin, Cefotaxime each 100% respectively and Gentamicin(66.7%).Pseudomonas aeruginosa was highly sensitive to Amikacin(100%), Gentamicin(100%), Ciprofloxacin(100%), Piperacillin-Tazobactum(100%), Imipenem(100%) and were highly resistant to Ceftazidime(100%). Acinetobacter baumanii isolate was highly sensitive to Tetracycline (100%), Piperacillin-Tazobactum (100%), Imipenem (100%) and

were highly resistant to Amikacin (100%), Gentamicin (100%), Ciprofloxacin (100%), Cotrimoxazole (100%) and Ceftazidime (100%).

In the present study, Gram positive organisms obtained were predominantly from patients from Intensive Care Units (42.9%) and (28.6%) were from patients from Medical wards. Gram negative organisms obtained were predominantly from Medical wards (80%) and remaining from Intensive Care Units (20%).

In this study, the co-morbidities and risk factors associated in patients with community acquired bloodstream infections were Diabetes mellitus (75%), Hypertension (33.3%), smoking (25%), anaemia (58.3%), and alcoholism (33.3%). Of which, the predominant risk factors were Diabetes mellitus (75%) and anaemia (58.3%) in this study. The study by Chung- Ting Chen et al[60] also showed that diabetes mellitus was the predominant risk factor associated with community acquired bloodstream infection.

In our study, the primary system involved in community acquired bloodstream infection patients were respiratory system (66.7%) followed by intraabdominal infections (16.7%), renal involvement (8.3%), skin and soft tissue infections (8.3%). Respiratory tract infection (66.7%) was the predominant source of infection for bacteraemia in the present study. Similar findings were observed in the study of J. Goncalves- Pereira et al[56] (2013), with respiratory system being the predominant primary source involved (60.6%). In Pedersen G et al[1] study, the commonest source of infection was the urinary tract (29%).

Molecular characterization of resistant isolates were done using polymerase chain reaction (PCR) which showed the presence of bla TEM and bla CTX-M genes, that confirmed ESBL producers among the Escherichia coli isolates and similarly, the presence of mecA gene confirmed methicillin resistant Staphylococcus aureus. Luzzaro F et al,[62] in his study found that the most prevalent ESBL producing Gram negative organism was found to be Escherichia coli and TEM- type ESBLs were found to be the most prevalent enzymes (45.4%).According to the study by Rossolini GM et al,[63] the CTX-M-type ESBLs had undergone a rapid and global spread in Enterobacteriaceae recently. In Mario Tumbarello et al[64] study, the predominantly isolated ESBL genes were bla CTX-M (36.5%) followed by bla TEM gene (28.7%).Nagat Sobhy et al[65] study emphasized that the identification of the mecA gene is the most reliable method for detecting the MRSA isolate.

Biomarkers of sepsis are very useful both in diagnosis and prognosis, also in assessing the severity of infection. In the present study, inflammatory markers, C-reactive protein (CRP) and Interleukin-6(IL-6) were tested and correlated with blood culture positivity (Statistically significant with a p value of 0.01).

All the serum samples which were collected from patients with evidence of sepsis were tested for C-reactive protein concentration using latex slide agglutination method. The concentration of CRP was found elevated in patients with positive blood culture (CRP conc. ranged between 190-400mg/l). 62 samples (52.5%) showed the CRP concentration range between 11-70mg/l, 30 samples

(25.4%) were in the range between 71-130mg/l, 14 samples (11.9%) were in the concentration range between 131-190mg/l and remaining 32 samples (21.3%) were in the range between 6-10mg/l.

Serum samples were tested for CRP, on the day of sample collection itself; hence results were obtained prior to blood culture report. 12 samples (10.2%) denoted a higher CRP concentration range between 190-400mg/l and these samples also showed blood culture positivity, which indicated that detection of CRP is useful as an early biomarker of sepsis.

CRP concentration range among Gram positive organisms were found between 190-200mg/l and among Gram negative organisms, the CRP concentration range was found between 190-400mg/l. There was no significant difference in CRP concentration range between Gram positive and Gram negative organisms were found. Among the resistant strains, CRP concentration of MRSA isolate was 192mg/l and the concentration range among ESBL producers were in the range between 300-400mg/l.

Also the same serum samples were tested for interleukin-6 levels by using sandwich ELISA method and all the culture positive cases showed higher concentration of interleukin -6 i.e. above 200pg/ml. 58 samples (59.2%) belonged to the concentration range between 70-120pg/ml, 28 samples (28.6%) were in the range between 12-60pg/ml and 52 samples (34.7%) showed the concentration range between 3-11pg/ml.

Interleukin-6 concentration levels among Gram positive organisms ranged between 300-900pg/ml and regarding Gram negative organisms, among Escherichia coli isolates, the concentration range was between 900-1200pg/ml. The concentration level of IL-6 in Pseudomonas aeruginosa isolate detected was found to be (1000-1200pg/ml) and for the Acinetobacter baumannii isolate identified, the IL-6 concentration level was found to be (600-800pg/ml).According to Ryuzoabe et al [66] (2010) study, the IL-6 levels and the CRP concentrations were found to be significantly elevated in sepsis due to Gram negative infections when compared to Gram positive infections suggesting a different immunomodulatory response. Among the resistant strains isolated, the interleukin-6 concentration for MRSA strain was found to be 826pg/ml and among the ESBL producers isolated, the concentration range was found between 900-1200pg/ml.

In this present study, out of 12 patients detected with community acquired bloodstream infections, 9(75%) recovered and 3(25%) expired. In the study by Sigauque B et al, community-acquired bacteraemia associated mortality accounted for 21% of hospital deaths. In the study by Valles J et al, [67] mortality rate in community- acquired bacteraemia was found to be 41.5%.Hence, this showed that the mortality rate in community acquired bloodstream infection/ sepsis could range between 21-42%. Among the recovered group of patients, the CRP concentration range was between 190-200mg/l and the interleukin-6 levels in these patients were in the range between 400-800pg/ml. Among 3 patients who

expired, their CRP concentration was in the range between 300-400mg/l and the IL-6 concentration ranges between 900-1200pg/ml.

Also in this study, out of 138 patients, who fulfilled the SIRS criteria clinically, but were culture negative, 136 patients (98.6%) got recovered and 2 patients (1.4%) expired. Among 136 patients who recovered, the CRP concentration range was found between 6-50mg/l and the interleukin-6 level was in the range between 3-60pg/ml. Among the 2 patients who expired, their CRP concentration range was between 131-190mg/l and the interleukin-6 concentration range was between 70-120pg/ml.

In the present study, CRP total positives were found to be 118(78.7%) and the total negatives were found to be 32(21.3%) with CRP cut- off being 6mg/l. IL-6 levels total positives were found to be 103(68.7%) and the total negatives were found to be 47(31.3%) with IL-6 cut- off value considered as 10pg/ml. According to Martin Hoenigl et al[68] study, the sensitivity of IL-6 with the recommended cut-off of 10pg/ml was more promising (sensitivity 99.6%). In another study by Hassan Boskabadi et al, [69] serum IL-6 at a cut-off value of 10pg/ml had a higher sensitivity 92.5% and positive predictive value of 97%. Hence, this cut-off value for IL-6 been chosen for this present study.

In this study, IL-6 was found to be both sensitive and specific than CRP as early sepsis marker statistically. Sensitivity of IL-6 was found to be 83% and specificity was 84%(95%confidence interval for sensitivity- lower limit 75% and the upper limit 89% and for specificity- lower limit 66% and the upper limit

94%)in CRP positive suspected sepsis cases. In the study by Noor MK et al,[70] IL-6 level was raised with high sensitivity 77% and specificity 74% in CRP positive suspected sepsis cases and hence, concluded in this study that, IL-6 is a very early marker of sepsis compared to CRP.

In this study, the sensitivity and specificity was confirmed by calculating positive predictive value of 95% and a negative predictive value of 57% (95% confidence interval for positive predictive value- lower limit 88% and the upper limit 98% and for negative predictive value- lower limit 42% and the upper limit 71%).

Further an ROC curve was plotted and obtained which showed the area under the curve was greater for IL-6(67%) than CRP (61%). This proved that IL-6 was found as a better early sepsis marker than CRP.

Also, statistically the median estimated IL-6 level by which the mortality in patients with community acquired sepsis could be predicted was found to be 1180pg/ml according to this study and the mean estimated IL-6 level was found to be 1040pg/ml. Pierre Damas et al[71] study found that the mortality rate increased significantly in the patients with IL-6 serum levels above 1000pg/ml which correlates well with the present study. Similarly, the median estimated CRP concentration by which the mortality in patients with community acquired sepsis could be predicted was found to be 384mg/l and the mean estimated CRP concentration was found to be 350mg/l. Shungo Yamamoto et al[72] study analysed that serum CRP levels >= 150mg/l was an independent predictor of death.

Summary

SUMMARY

The study was done during a period of 1year from March 2017- February 2018 at Institute of Microbiology in collaboration with the Institute of Internal Medicine and Surgery. The study group included 150 patients in the age group > 18yrs with clinical suspicion of sepsis admitted within 48hrs in Medical, surgical wards and Intensive Care Units at Rajiv Gandhi Government General Hospital.

The presenting symptoms in the present study were fever, cough, dyspnoea, abdominal pain, vomiting, dysuria, ulcerative skin lesions, swelling legs, malena/bleeding disorders, altered sensorium, seizures.

Blood culture was done in these 150 patients prior to antibiotic administration, of which community acquired bloodstream infection was detected in 12patients (8%).

Majority of patients 6(50%) with community acquired bloodstream infection belonged to the age group between 51-60 yrs and males were predominantly affected 9(75%) than females 3(25%).

The organisms isolated were 58% Gram positive organisms and 42% Gram negative organisms.

Majority of clinical isolates were from Medical unit (both general wards and Intensive care unit). Gram positive isolates obtained were predominantly from

Intensive Care Unit (42.9%) and Gram negative isolates obtained were predominantly from general medical wards (80%).

Among the Gram positive organisms- 85.7% were Methicillin sensitive Staphylococcus aureus (MSSA) and 14.3% were Methicillin resistant Staphylococcus aureus (MRSA).

Among the Gram negative organisms, 60% Escherichia coli, 20% Acinetobacter baumannii and 20% Pseudomonas aeruginosa were isolated.

Antimicrobial susceptibility testing was done for these organisms which detected 60% ESBL producing Escherichia coli isolates using both screening test and phenotypic confirmatory test as per CLSI guidelines. Also, Staphylococcus aureus isolates were tested for Methicillin resistance by disk diffusion method using cefoxitin (30µg) disk and 14.3% Methicillin resistant Staphylococcus aureus isolate was identified and then confirmed by PCR, by detecting mecA gene. Gram positive organisms were found highly sensitive to Erythromycin, Vancomycin, Linezolid each 100% respectively and the Gram negative organisms were found highly sensitive to Amikacin, Tetracycline each 100% respectively.

Vancomycin sensitivity in Gram positive organisms tested using Vancomycin screen agar and E –strip test found to be sensitive to Vancomycin.

Antimicrobial resistant genes for the resistant isolates were detected using PCR. bla TEM and bla CTX-M genes were found positive among ESBL

producing Escherichia coli isolates and mecA gene was found to be positive in Methicillin resistant Staphylococcus aureus isolate.

Diabetes mellitus (75%) and anaemia (58.3%) were found to be predominant risk factors associated in patients with community acquired bloodstream infection in this study.

Respiratory system was the predominant primary system involved (66.7%) in patients with community acquired bloodstream infections according to this study.

Inflammatory markers CRP and IL-6 concentrations were tested using latex slide agglutination method and sandwich ELISA method respectively and correlated with blood culture positivity. Where CRP and IL-6 levels were both found to be positive and were significantly elevated in blood culture positive samples (CRP concentration ranged between 190-400mg/l and IL-6 concentration was >= 200pg/ml).

IL-6 was found to be both sensitive and specific than CRP as early sepsis marker statistically with sensitivity 83%(95% CI- 75%-89%), specificity 84%(95% CI-66%-94%), positive predictive value 95%(95% CI-88%-98%) and the negative predictive value 57%(95% CI-42%-71%) in CRP positive suspected sepsis cases. Also ROC curve plotted showed, area under the curve for IL-6 (67%) was more than CRP (61%). This proved, IL-6 as a better early sepsis marker than CRP as per this study.

The clinical outcome of the patients with community acquired bloodstream infection, (75%) recovered and (25%) expired. Among the patients who expired, CRP and IL-6 levels were significantly raised (CRP concentration ranged between 300-400mg/l, IL-6 concentration ranged between 900-1200pg/ml) than the recovered group (CRP concentration ranged between 190-200mg/l, IL-6 concentration ranged between 400-800pg/ml).

In this study, statistically the median estimated IL-6 level by which the mortality in patients with community acquired sepsis could be predicted was found to be 1180pg/ml and the median estimated CRP concentration was found to be 384mg/l.

Correlation of inflammatory markers (CRP and IL-6) in this study was statistically significant with a p value of 0.01.

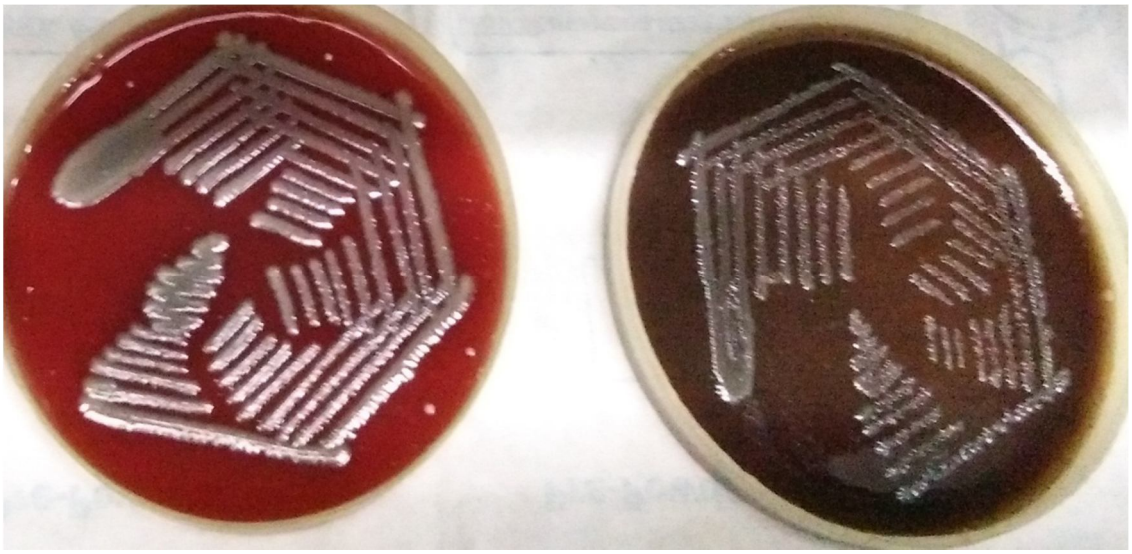
Conclusion

CONCLUSION

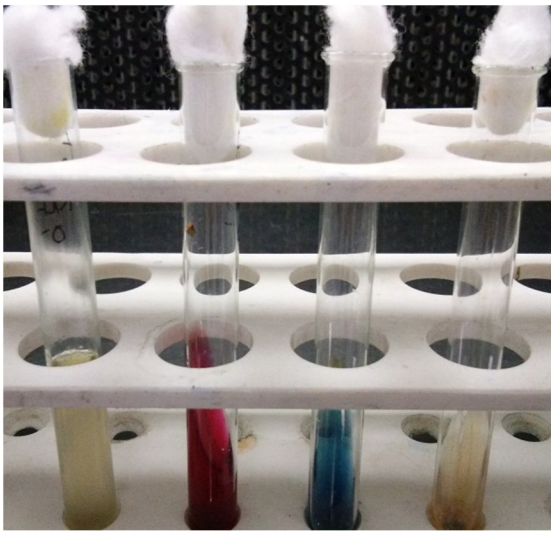
- Community acquired bloodstream infections are rising as a major health problem in upcoming years due to the emergence of antimicrobial resistant organisms which were once confined to hospital settings are now a potential threat in the community settings as well like ESBL producing Enterobacteriaceae, MRSA etc...
- Hence, these antimicrobial resistant strains should be promptly identified through proper surveillance. Molecular characterization of resistant pathogens helps in tracking the spread of antimicrobial resistance in the community.
- Also, appropriate antibiotic policy and preventive strategies has to be framed to curtail the spread of these antimicrobial resistant strains in the community settings.
- Inflammatory markers such as CRP, IL-6 have a major role in early sepsis detection. Of which, IL-6 is found to a better early marker of sepsis than CRP which reveals positivity within 24hrs of onset of sepsis than the conventional blood cultures. But still, blood culture remains the confirmatory and gold standard method in the detection of sepsis.

Colour Plates

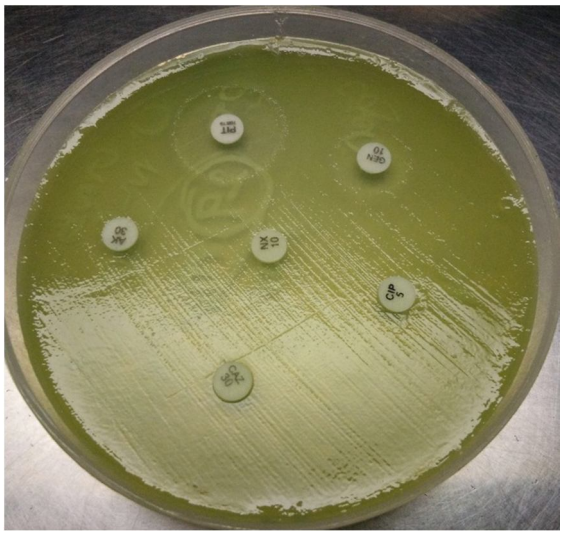
1.Acinetobacter baumannii colony growth on MacConkey agar plate



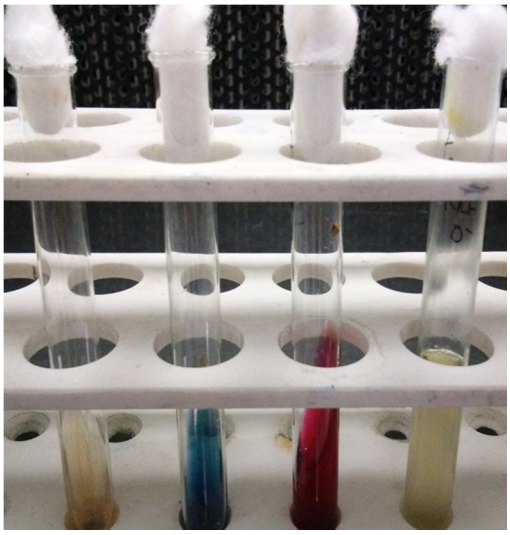
2.Acinetobacter baumannii colony growth on blood agar & chocolate agar plate



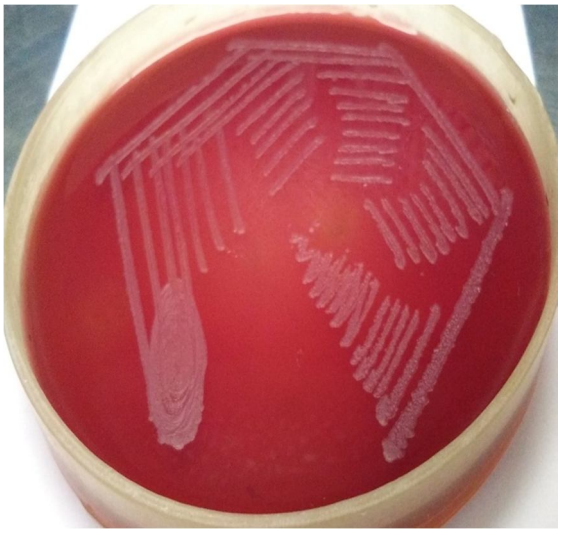
3.Biochemical reactions of Acinetobacter baumannii



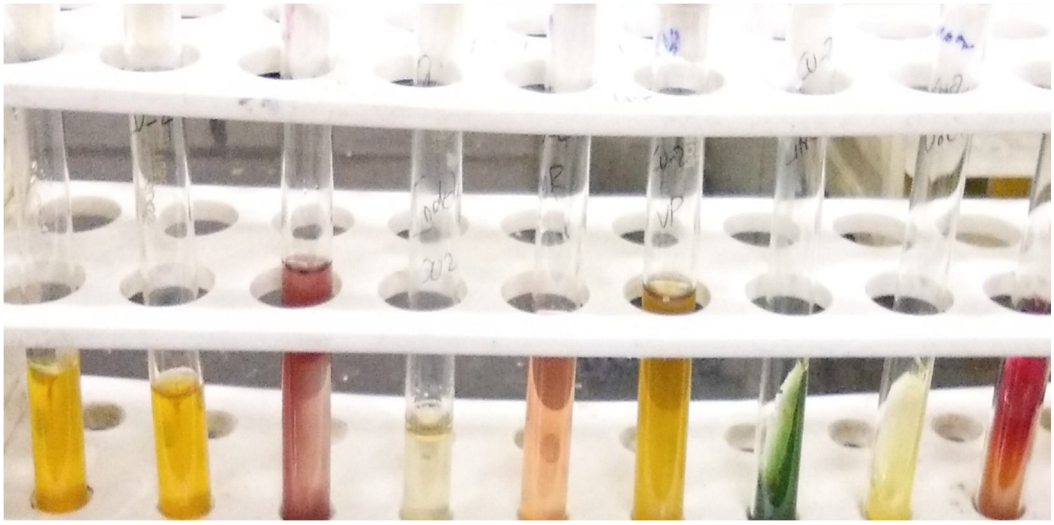
4.Pseudomonas aeruginosa pigmented colony on Mueller Hinton agar plate



5.Biochemical reactions of Pseudomonas aeruginosa



6.E.coli colony growth on MacConkey agar plate

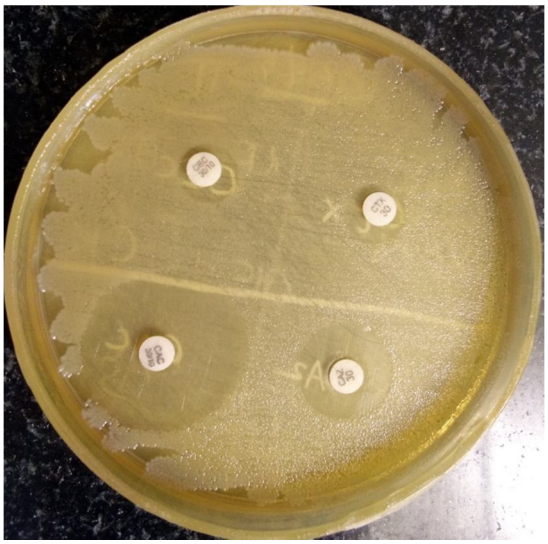


7.Biochemical reactions

8.Staphylococcus aureus colony growth on MacConkey agar plate

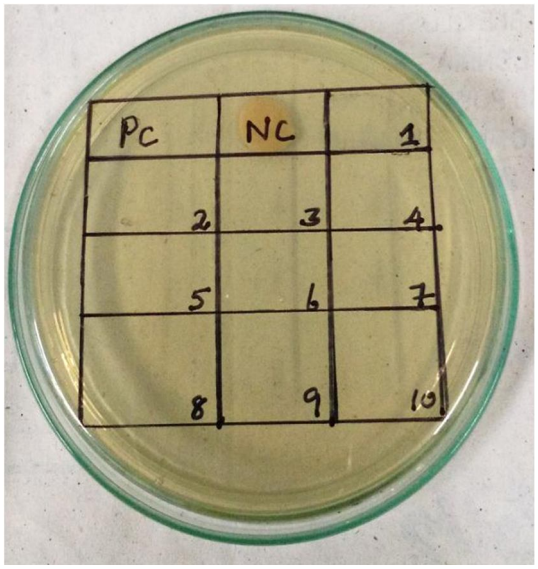
9.Staphylococcus aureus colony growth on blood agar plate showing beta hemolysis

10.Staphylcoccus aureus colony growth on chocolate agar plate

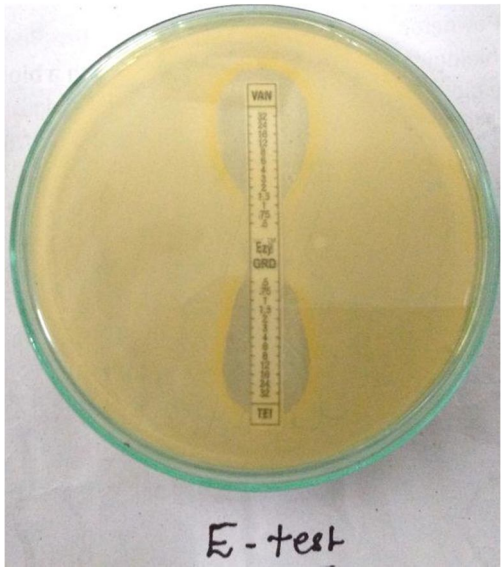


11.ESBL detection pic 1

12.ESBL detection pic 2

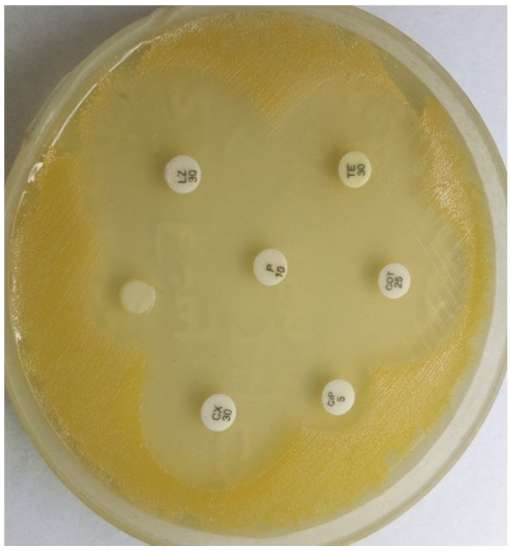


13.Vancomycin Screen agar

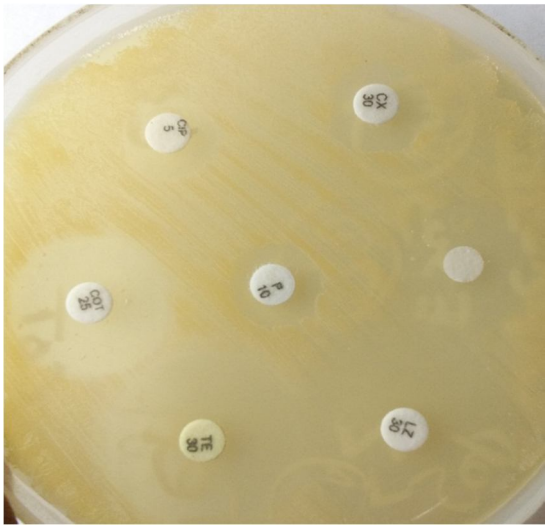


14.E test

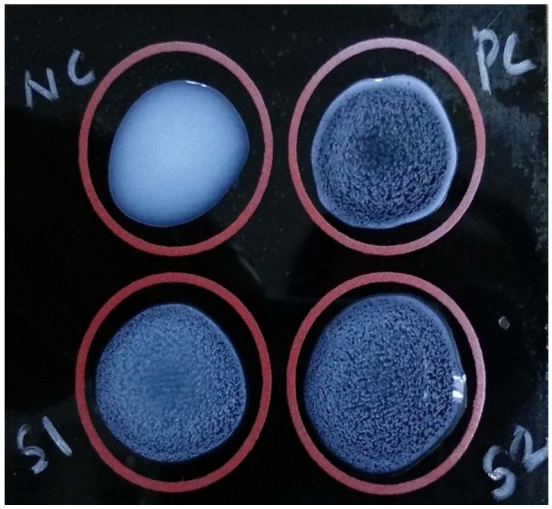
15.Methicillin sensitive Staphylococcus aureus-pic1



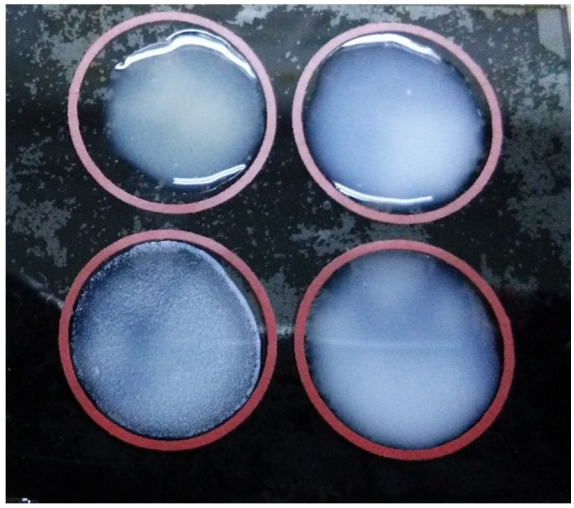
16.Methicillin sensitive Staphylococcus aureus -pic2



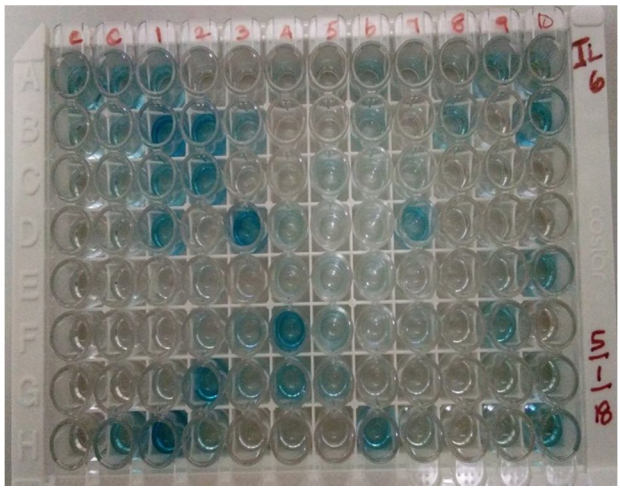
17. Methicillin resistant Staphylococcus aureus



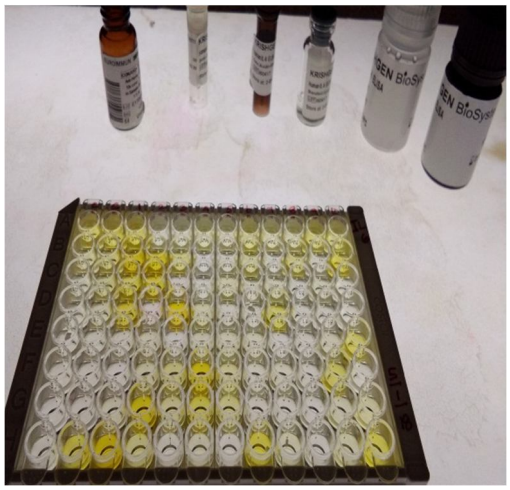
18.CRP detection- latex agglutination test- pic 1



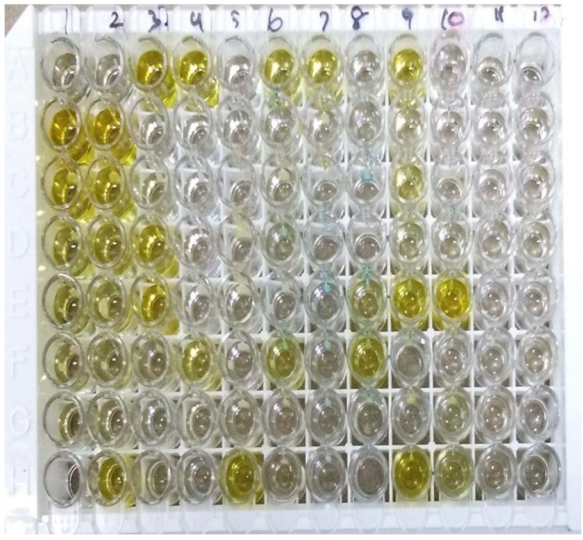
19.CRP detection- latex agglutination test- pic 2



20.IL-6 picture 1

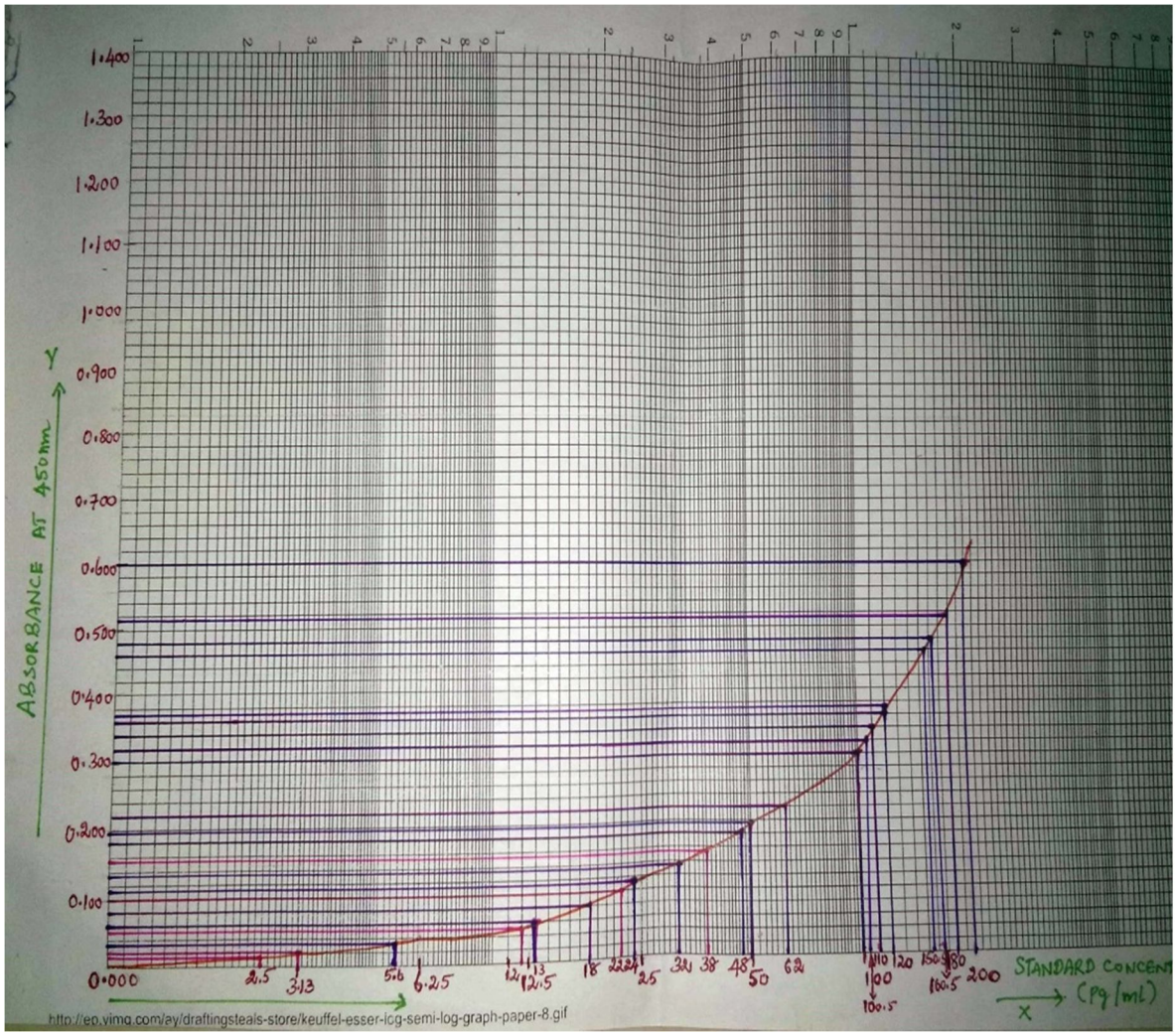


21.IL-6 picture 2

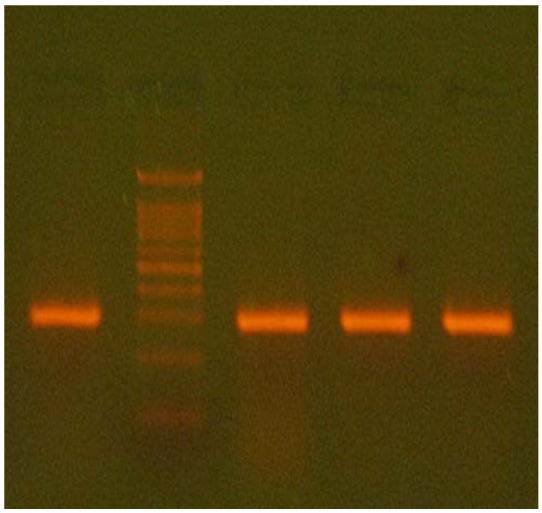


22.IL6 picture 3

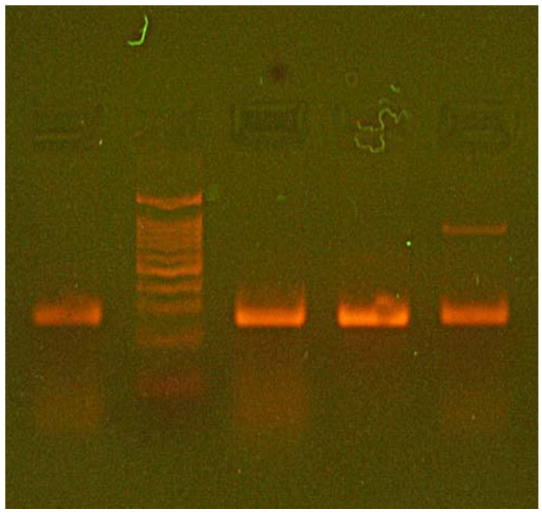
23.ELISA washer



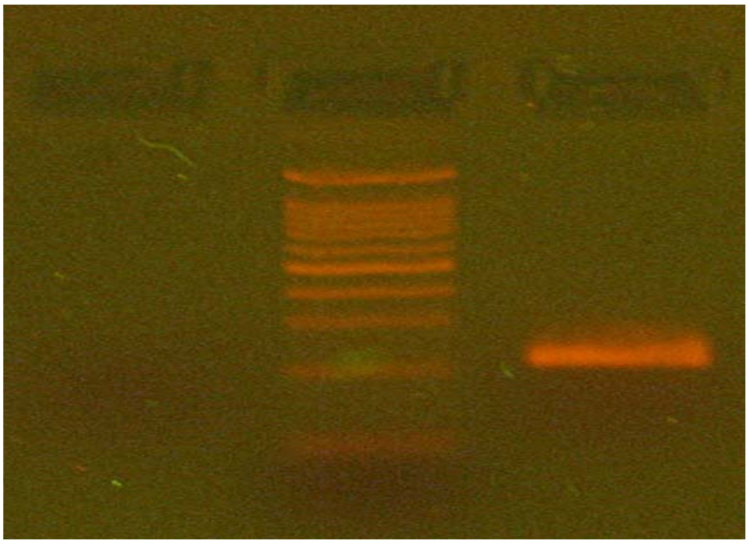
24.IL-6 Graph plotted on a semilog paper



25.bla CTX-M-ladder-S6,S7,S8



26.bla TEM-LADDER-S6,S7,S8



27.NTC-Ladder-mecA

Bibliography

BIBLIOGRAPHY

1. Pedersen G, Schonheyder H.C., Sorensen H. T. Source of infection and other factors associated with case fatality in community- acquired bacteremia- a Danish population- based cohort study from 1992 to 1997. Clin Microbiol Infect 2003; 9:793-802.
2. Larry G. Reimer, Michael L. Wilson, Melvin P. Weinstein. Update on detection of bacteremia and fungemia. Clinical Microbiology Reviews, 1997, 10:444-465.
3. J. Rodriguez- Bano, M.D. Lopez- Prieto, M.M. Portillo, et al. Epidemiology and clinical features of community- acquired, healthcare- associated and nosocomial bloodstream infections in tertiary-care and community hospitals. Clin Microbiol Infect 2010; 16: 1408-1413.
4. Garner JS, Jarvis WR, Emori TG, et al. CDC definitions for nosocomial infections. Am J Infect Control 1988; 16:128-40.
5. Yardena Siegman – Igra, Boaz Fourer, Ruth Orni- Wasserlauf, et al. Reappraisal of community- acquired bacteremia: A proposal of a New Classification for the spectrum of acquisition of bacteremia. Clinical infectious diseases 2002 ; 34:1431-9.
6. Johann D.D.Pitout, Patrice Nordmann, Kevin B. Laupland et al. Emergence of Enterobacteriaceae producing extended- spectrum β- lactamases (ESBL) in the community. J antimicro chemo. 2005; 56: 52-59.
7. Roger C Bone, Robert A. Balk, Frank B. Cerra, et al. Definitions for sepsis and organ failure and guidelines for the use of Innovative Therapies in Sepsis. Chest 1992; 101:1644-55.
8. Mitchell M.Levy, Mitchell P.Fink, John C. Marshall, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions conference. Intensive Care Medicine 2oo3; 29:530-538.
9. Mervyn Singer, Clifford S. Deutschman, Christopher Warren Seymour, et al. The Third International Consensus definitions for sepsis and septic shock (sepsis-3). JAMA 2016; 315:801-810.

10. Mark B. Pepys, Gideon M. Hirschfield. C-reactive protein: a critical update. J Clin Invest. 2003; 111:1805-1812.

11. Nora Hofer, Eva Zacharias, Wilhelm Muller, et al. An update on the use of C-reactive protein in early – onset neonatal sepsis: current insights and New Tasks. Neonatology 2012; 102:25-36.

12. James D. Faix. Biomarkers of sepsis. Crit Rev Clin Lab Sci 2013; 50:23-36.

13. Debasree Banerjee, Mitchell M. Levy (2017) Sepsis definitions In:Nicholas S. Ward, Mitchell M. Levy (eds.). Sepsis- Definitions, Pathophysiology and the challenge of Bedside Management. Respiratory medicine, Part I, Springer, p 7-24.

14. Duane J.Funk, Joseph E.Parrillo, Anand Kumar. Sepsis and Septic shock history. Crit Care Clin 2009; 25:83-101.

15. Bone RC, Fisher CJ Jr, Clemmer TP, et al. Sepsis syndrome: a valid clinical entity, Methylprednisolone Severe Sepsis Study Group. Crit Care Med 1989; 17:389-93.

16. Vincent JL. (2001)Definitions: Sepsis versus SIRS In: Eichacker P. Q. Pugin J.(eds.).Evolving concepts in sepsis and septic shock. Perspectives on critical care Infectious Diseases, vol 2. Springer, Boston, MA. p 17-25.

17. Fethi Gul, Mustafa Kemal Arslantas, Ismail Cinel, et al. Changing Definitions of Sepsis. Turk J Anaesthesiol Reanim 2017; 45:129-38.

18. Christopher W. Seymour, Vincent X. Liu, Theodore J. Iwashyna, et al. Assessment of clinical criteria for sepsis for the Third International Consensus Definitions for sepsis and septic shock(sepsis-3). JAMA 2016; 315:762-774.

19. Kelly Roveran Genga, James A. Russell. Update of sepsis in the Intensive Care Unit. J Innate Immun 2017; 9:441-455.

20. Paul E. Marik, Abdalsamih M. Taeb. SIRS, q SOFA and new sepsis definition. J. Thorac Dis.2017; 9:943-945.

21. Alan E. Jones, Stephen Trzeciak, Jeffrey A. Kline. The Sequential Organ Failure Assessment Score for predicting outcome in patients with severe sepsis and evidence of hypoperfusion at the time of emergency department presentation. Crit Care Med 2009; 37:1649-1654.

22. N. Deborah Friedman, Keith S. Kaye, Jason E. Stout, et al. Health care- associated bloodstream infections in adults: A reason to change the accepted definition of community- acquired infections. Ann Intern Med. 2002; 137:791-797.

23. Daniel Pilsgaard Henriksen, Anton Pottegard, Christian B. Laursen, et al. Risk factors for hospitalization due to community- acquired sepsis- A population- based case- control study. PLoS One. 2015; 10:e0124838.

24. Dae Won Park, Byung Chul Chun, June Myung Kim, et al. Epidemiological and clinical characteristics of community- acquired severe sepsis and septic shock: A prospective observational study in 12 university hospitals in Korea. J Korean Med Sci 2012; 27:1308-1314.

25. Chien- Chang Lee, Shey- Ying Chen, I- Jing Chang, et al. Comparison of clinical manifestations and outcome of community- acquired bloodstream infections among the oldest old, elderly, and adult patients. Medicine 2007; 86:138-144.

26. K. B. Laupland, D.B. Gregson, W.W.Flemons, et al. Burden of community- onset bloodstream infection: a population- based assessment. Epidemiol. Infect. 2007; 135:1037-1042.

27. Kevin B. Laupland, Deirdre L. Church. Population- Based Epidemiology and Microbiology of community- onset bloodstream infections. Clin Microbiol Rev. 2014; 27:647-64.

28. Dr Jacqueline Deen, Lorenz Von Seidlein, Finn Andersen, et al. Community-acquired bacterial bloodstream infections in developing countries in south and southeast Asia: a systematic review. Lancet Infect Dis. 2012; 12: 480-487.

29. Elizabeth A Reddy, Andrea V Shaw, John A Crump. Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. Lancet Infect Dis. 2010; 10:417-432.

30. Eirini Christaki, Evangelos J Giamarellos- Bourboulis. The complex pathogenesis of bacteremia from antimicrobial clearance mechanisms to the genetic background of the host. Virulence 2014; 5:57-65.

31. Daniel G. Remick. Pathophysiology of sepsis. Am J Pathol. 2007; 170:1435-1444.

32. Brigitte Lamy, Sylvie Dargere, Maiken C. Arendrup. How to optimize the use of blood cultures for the diagnosis of bloodstream infections? A state- of-the Art. Front Microbiol. 2016; 7:697.

33. Emilio Bouza, Dolores Sousa, Marta Rodriguez- Creixems, et al. Is the volume of blood cultures still a significant factor in the diagnosis of bloodstream infections? J Clin Microbiol. 2007; 45:2765-2769.

34. T.J. Kirn, M.P. Weinstein. Update on blood cultures: How to obtain, process, report and interpret. Clin Microbiol Infect 2013; 19:513-520.

35. Pedro Povoa. C-reactive protein: a valuable marker of sepsis. Intensive Care Med 2002; 28:235-243.

36. Snjezana Rothkrantz-Kos, Maria P. J. Schmitz, Otto Bekers, et al. High- Sensitivity C-reactive protein methods examined. Clinical Chemistry 2002; 48:359-362.

37. Toshio Tanaka, Masashi Narazaki, Tadamitsu Kishimoto. IL-6 in inflammation, immunity and disease. Cold Spring Harb Perspect Biol. 2014; 6:a016295.

38. Peter C. Heinrich, Jose V.Castell, Tilo Andus. Interleukin-6 and the acute phase response. Biochem. J. 1990; 265:621-636.

39. Li S, Li J, Qiao Y, et al. Prevalence and invasiveness of community-acquired methicillin-resistant Staphylococcus aureus : a meta-analysis. Indian J Pathol Microbiol. 2014; 57: 418-22.

40. Niccolo Buetti, Andrew Atkinson, Jonas Marschall, et al. Incidence of bloodstream infections: a nationwide surveillance of acute care hospitals in Switzerland 2008-2014. BMJ open 2017; 7: e013665.

41. Shankar- Hari M, Harrison DA, Rubenfeld GD, et al. Epidemiology of sepsis and septic shock in critical care units:Comparison between sepsis-2 and sepsis-3 populations using a national critical care database. Br J Anaesth 2017; 119:626-636.

42. Florian B Mayr, Sachin Yende , Derek C Angus. Epidemiology of severe sepsis. Virulence. 2014; 5:4-11.

43. T J Evans. Immunotherapy of sepsis. J. Med.Microbiol. 1993; 38:237-239.

44. Schoenberg MH, Weiss M, Radermacher P. Outcome of patients with sepsis and septic shock after ICU treatment. Langenbecks Arch Surg. 1998; 383:44-8.

45. CDC- Statistical reports on the incidence, prevalence and mortality rates of sepsis, 2018.

46. Artero A, Zaragoza R, Camarena J J, et al. Prognostic factors of mortality in patients with community- acquired bloodstream infection with severe sepsis and septic shock. J Crit Care. 2010; 25:276-81.

47. J. Rello, M. Kollef, E.Diaz, A. Rodriguez(Eds). Infectious Diseases in critical care (2007) 2nd edition, springer, P-300.

48. CDC Report calls for sepsis education , prevention-AAFP, 2016.

49. Bailey & Scott's Diagnostic Microbiology 13th edition, P-866-869.

50. Mackie & McCartney Practical Medical Microbiology 14th edition, P-254-256.

51. Koneman's Color atlas and textbook of Diagnostic Microbiology 7th edition, P-316-385.

52. Clinical and Laboratory Standards Institute (CLSI) M100 27th edition Performance Standards for antimicrobial susceptibility testing.

53. Tufail Soomro, Shiyam Sunder Tikmani, Sumera Aziz Ali. Frequency and Etiology of community- acquired bloodstream infection in hospitalized febrile children. J Med Diagn Meth 2016; 5:217.

54. Sigauque B, Roca A, Mandomandol, et al. Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. Pediatr Infect Dis J. 2009; 28:108-13.

55. Nathan I. Shapiro, Richard E. Wolfe, Sharon B. Wright, et al. Who needs a blood culture? A prospectively derived and validated prediction rule. J Emerg Med 2008; 35:255-264.

56. J. Goncalves Pereira, P.R. Povoa, C. Lobo, et al. Bloodstream infections as a marker of community-acquired sepsis severity. Results from the Portuguese Community-acquired sepsis study (SACiUCI study). Clin Microbio Infect 2013; 19:242-248.

57. Ssali FN, Kamya MR, Wabwire-Mangen F, et al. A prospective study of community-acquired bloodstream infections among febrile adults admitted to Mulago Hospital in Kampala, Uganda. J Acquir Immune Defic Syndr Hum Retrovirol. 1998; 19:484-9.

58. Klevens RM, Morrison MA, Nadle J, et al.Invasive Methicillin resistant Staphylococcus aureus infections in the United States. JAMA 2007; 298:1763-1771.

59. Parkins MD, Gregson DB, Pitout JD, et al. Population-based study of the epidemiology and the risk factors for Pseudomonas aeruginosa bloodstream infection. Infection 2010; 38:25-32.

60. Chung-Ting Chen, Yung-Chih Wang, Shu-Chen Kuo, et al. Community-acquired bloodstream infections caused by Acinetobacter baumannii; A matched case-control study. j. jmii.2017; 02.004.

61. Quan J, Zhao D, Liu L, et al. High prevalence of ESBL- producing Escherichia coli and Klebsiella pneumoniae in community-onset bloodstream infections in China. J Antimicrob Chemother 2017; 72:273-280.

62. Luzzaro F, M. Mezzatesta, C. Mugnaioli, et al. Trends in production of extended spectrum beta-lactamases among Enterobacteria of medical interest: report of the second Italian nationwide survey. J. Clin. Microbiol. 2006; 44:1659-1664.

63. Rossolini G.M, M.M.D′ Andrea, C.Mugnaioli. The spread of CTX-M- Type extended spectrum β-lactamases. CLIN. Microbiol. Infect. 2008; 14(suppl.1): S33-S41.

64. Mario Tumbarello, Michela Sali, Enrico Maria Trecarichi, et al. Bloodstream infections caused by Extended- spectrum- β-lactamase-producing Escherichia coli: Risk factors for inadequate initial antimicrobial therapy. J Antimicrob Chemother. 2008; 52:3244-3252.

65. Nagat Sobhy, Fatma Aly, Ola Abd El Kader et al. Community-acquired methicillin-resistant Staphylococcus aureus from skin and soft tissue infections(in a sample of Egyptian population):analysis of mecA gene and Staphylococcal cassette chromosome.Braz J Infect Dis.2012; 16:426-431.

66. RyuzoAbe, Shgeto oda, Tomohito Sadahiro, et al. Gram-negative bacteremia induces greater magnitude of inflammatory response than Gram-positive bacteremia. Crit Care 2010; 14:R27.

67. Valles J, Rello J, Ochagavia A, et al. Community- acquired bloodstream infection in critically ill adult patients: Impact of shock and inappropriate antibiotic therapy on survival. Chest 2003; 123:1615-24.

68. Martin Hoenigl, Jasmin Wagner, Reihard B. Raggam, et al. Characteristics of Hospital-acquired and Community- onset bloodstream infections, South-East Austria. PLOS ONE 2014; 9:e104702.

69. Hassan Boskabadi, Gholamali Maamouri, Jalil Tavakol Afshari, et al. Evaluation of serum Interleukins- 6,8 and 10 levels as diagnostic markers of neonatal infection and possibility of mortality. Iran J Basic Med Sci. 2013; 16:1232-1237.

70. Noor MK, Shahidullah M, Mutanabbi M, et al. Comparison between CRP and IL-6 as early markers of neonatal sepsis. Mymensingh Med J 2008; 17(supp 2) S 72-6.

71. Pierre Damas, Didier Ledoux, Monique NYS, et al. Cytokine serum level during severe sepsis in Human IL-6 as a marker of severity. Ann Surg.1992; 215:356-362.

72. Shungo Yamamoto, Shin Yamazaki, T Sunehiro Shimizu, et al. Prognostic utility of serum CRP levels in combination with CURB-65 in patients with clinically suspected sepsis: A decision curve analysis. BMJ Open. 2015; 5:e007049.

Annexures

APPENDIX

ABBREVIATIONS

BHI broth	-	Brain heart infusion broth
MSSA	-	Methicillin sensitive Staphylococcus aureus
MRSA	-	Methicillin resistant Staphylococcus aureus
ESBL	-	Extended spectrum beta-lactamase
SIRS	-	Systemic inflammatory response syndrome
CRP	-	C-reactive protein
IL-6	-	Interleukin-6
PCR	-	Polymerase chain reaction
CLSI	-	Clinical and Laboratory Standards Institute
SD	-	standard deviation
ROC curve	-	Receiver Operating Characteristic Curve
INR	-	International Normalized Ratio

ANNEXURE – I

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr.A.Priyadharshini
Post Graduate in M.D. Microbiology
Institute of Microbiology
Madras Medical College
Chennai 600 003

Dear Dr.A.Priyadharshini,

The Institutional Ethics Committee has considered your request and approved your study titled **"A STUDY ON COMMUNITY ACQUIRED BLOODSTREAM INFECTIONS, MOLECULAR CHARACTERIZATION OF RESISTANT PATHOGENS AND CORRELATION WITH INFLAMMATORY MARKERS IN A TERTIARY CARE HOSPITAL"- NO.13012017 (IV).**

The following members of Ethics Committee were present in the meeting hold on **31.01.2017** conducted at Madras Medical College, Chennai 3

1.Dr.C.Rajendran, MD.,	:Chairperson
2.Dr.M.K.Muralidharan,MS.,M.Ch.,Dean, MMC,Ch-3	:Deputy Chairperson
3.Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3	: Member Secretary
4.Prof.B.Vasanthi,MD., Prof.of Pharmacology.,MMC,Ch-3	: Member
5.Prof.S.Suresh,MS, Prof. of Surgery,MMC,Ch-3	: Member
6.Prof.N.Gopalakrishnan,MD,Director,Inst.of Nephrology,MMC,Ch	: Member
7.Prof.S.Mayilvahanan,MD,Director, Inst. of Int.Med,MMC, Ch-3	: Member
8.Tmt.J.Rajalakshmi, JAO,MMC, Ch-3	: Lay Person
9.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary – Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

ANNEXURE – II

PROFORMA

- ⊡ Name:
- ⊡ Age/gender:
- ⊡ IP no:
- ⊡ Date of admission:
- ⊡ Presenting complaints:
- ⊡ Past history:
- ⊡ Personal history:
- ⊡ Treatment history:
- ⊡ Travel history:
- ⊡ Investigations:

ANNEXURE - III

CONSENT FORM

STUDY TITLE:

A STUDY ON COMMUNITY ACQUIRED BLOODSTREAM INFECTIONS, MOLECULAR CHARACTERIZATION OF RESISTANT PATHOGENS AND CORRELATION WITH INFLAMMATORY MARKERS IN A TERTIARY CARE HOSPITAL.

I……, hereby give consent to participate in the study conducted by **Dr.A.PRIYADHARSHINI,** Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my clinical Sample (blood) for further investigations. I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal.

Signature/ Thumb impression Of the patient/ relative

Place

Date

Patient Name & Address:

Signature of the investigator:

Signature of guide:

ANNEXURE - IV

INFORMATION SHEET

STUDY TITLE:

A STUDY ON COMMUNITY ACQUIRED BLOODSTREAM INFECTIONS, MOLECULAR CHARACTERIZATION OF RESISTANT PATHOGENS AND CORRELATION WITH INFLAMMATORY MARKERS IN A TERTIARY CARE HOSPITAL.

INVESTIGATOR : **Dr.A.PRIYADHARSHINI,**
Post Graduate,
Institute of Microbiology,
Madras Medical College,
Chennai - 600003

GUIDE : **Dr. C.P.RAMANI, M.D.,**
Professor of Microbiology,
Institute of Microbiology,
Madras Medical College,
Chennai - 600003

In this study, I am going to collect blood samples from patients with informed consent who have been hospitalized within 48 hours with sepsis and process them to identify the causative agents of community acquired bloodstream infections.150 patients will be included in this study only after getting informed consent. Also, in this study the multidrug resistant pathogens will be characterized by phenotypic and genotypic methods. Early biomarkers of sepsis such as C-reactive protein, Interleukin-6 determined and correlated with blood culture positivity.

This study is entirely voluntary and patient can withdraw any time from this study. Extra cost will not be incurred to the patients in this study. Any doubt regarding this study will be clarified. Results of this study will be published. In case of any doubt please contact, Dr.A.PRIYADHARSHINI, CELL: 8903792132.

MASTER CHART

Sample Number	Name	Age(yrs)	Sex	Ward/ Ip No	Date of admission	Chief complaints	Past history	Relevant Examination Findings	Hb	Tc	Dc	ESR	platelet count	MCV	MCH	MCHC	Other Investigations	Culture Identification	CRP mg/l	IL-6(pg/ml)	**outcome**
1	sumathi	57	f	121/81469	6.3.17	fever, vomiting, pain abdomen	RHD-Operated, old CVA	febrile, BP-80/60 mmHg, PR-106/mt, RR-22/mt, dehydration+ P/A-epigastric tenderness+ L hemiparesis	11.5	14700	72%19%1%0%1%	22	160,000	85.7	27.3	32.9	scrub typhus Igm-positive	NG	48	96	recovered
2	parthiban	19	m	113/82293	7.3.17	fever, cough, joint pains	nil	febrile,BP-90/60mmHg,PR-102/mt,CVS/RS-NAD	14.5	4900	78%22%0%1%0%	14	138,000	85.2	27.3	32	-	NG	12	72	recovered
3	sampath	51	m	212/82316	8.3.17	fever,cough,dyspnoea, myalgia	nil	febrile,BP-80/60mmHg, dyspnoeic,tachypnoeic, PR-108/mt,RR-26/mt, eschar/ulcer-R scrotum	15.8	13900	76%21%1%1%0%	16	80,000	88.2	31	35.1	CT chest-L pleural effusion, IgM Dengue positive	NG	48	32	recovered
4	Balaji	57	m	113/82327	9.3.17	fever,vomiting,myalgia	nil	febrile,BP-100/70mmHg, dehydration+,PR-102/mt, P/A-splenomegaly+	10.6	3800	70%18%0%0%0%	12	202,000	79.5	26.5	32.1	P.S-P.vivax USG abd-splenomegaly	NG	6	3.13	recovered
5	Baskar	23	m	113/82352	9.3.17	fever,myalgia	nil	febrile,BP-90/60mmHg, PR-98/mt,dehydration+	12.2	5000	74%32%0%1%0%	18	75000	86.2	28.1	33.1	IgM Dengue positive	NG	12	54	recovered
6	selvamani	21	m	113/82370	10.3.17	fever,myalgia	nil	febrile,PR-108/mt,BP-90/70 mmHg,dehydration+,	11.9	3400	68%31%1%0%0%	10	68,000	82.4	32.1	34.2	IgM Dengue positive	NG	12	48	recovered
7	Rajeswari	32	f	212/82245	13.3.17	fever,pain abdomen, vomiting,cough,dyspnoea	nil	febrile,PR-106/mt, BP-80/60mmHg,rashes,abd. Distension,dyspnoeic, tachypnoeic,RR-28/mt, RS-R lung base reduced breath sounds	11.2	15100	80%29%1%1%0%	18	59000	85.4	28.9	33.9	IgM Dengue positive USG abd-R pleural effusion with mod. Ascitis	NG	24	32	recovered
8	Sarathy	20	m	125/82863	16.3.17	fever,vomiting,altered sensorium	nil	febrile,BP-100/60mmHg, drowsiness,dehydration+ PR-102/mt	12.1	13000	78%21%0%0%0%	14	166,000	86.2	28.7	33.3	hyponatremia	NG	6	4.2	recovered
9	Muniyandi	21	m	212/83372	17.3.17	fever,vomiting,pain abdomen,bleeding gums	nil	febrile,BP-80/60mmHg, PR-96/mt,dehydration+	10.2	4300	65%24%0%0%0%	12	60,000	78.1	27.6	32.1	IgM Dengue positive	NG	12	46	recovered
10	Roja	35	f	123/81858	19.3.17	fever,myalgia	nil	febrile,dehydration, PR-104/mt,BP-100/70mmHg	12.3	8600	72%22%1%0%0%	16	120,000	86.5	28.2	32.6	PS-P.vivax	NG	6	6.25	recovered
11	Syed	23	m	145/83036	24.3.17	fever,vomiting,myalgia	nil	febrile,BP-90/60mmHg, dehydration+	12	9600	76%28%0%1%0%	12	96000	86.2	28.2	32.6	IgM Dengue positive	NG	96	24	recovered
12	Yasodha	35	f	121/83485	10.4.17	fever,vomiting,pain abdomen	nil	febrile,BP-100/70mmHg, PR-102/mt,dehydration+	11.3	8400	74%22%0%0%1%	16	222,000	92.8	31.5	33.9	-	NG	6	6.25	recovered
13	Prema	55	f	121/83553	14.4.17	fever,headache, myalgia	nil	febrile,BP-90/60mmHg, PR-104/mt	12	7900	78%29%1%0%0%	18	169,000	83.4	28.4	34	-	NG	6	5.6	recovered
14	Ellammal	75	f	124/2161	16.4.17	fever,dyspnoea,altered sensorium,vomiting	DM-12yrs,HT-10yrs	febrile,drowsy,PR-110/mt BP-80/60mmHg,b/l basal lung crepts,dehydration+	11.2	14100	82%28%0%1%1%	28	194,000	88.2	26.5	30.1	urine acetone-positive urineRE-albumin 2+	NG	24	76	recovered
15	Usman	21	m	212/3000	18.4.17	fever,vomiting,syncope	nil	febrile,PR-106/mt, BP-100/70mmHg,dehydration	13.8	5300	72%24%0%1%0%	14	150,000	81.6	29.6	36.2	-	NG	12	3	recovered

16	Hemavathy	68	f	122/2875	22.4.17	fever,inc.freq.of micturition,dyspnoea, altered sensorium	HT-15yrs,old CVA	febrile,drowsy,PR-108/mt, dehydration,spo2-88%, BP-160/100mmHg	10.6	16500	80%28%0%1%0%	12	264,000	88.9	29.5	30.2	hyponatremia MRI brain-Periventricular white matter hypodensities	NG	12	6.25	recovered
17*	Vasantha	61	f	212/3158	26.4.17	fever,vomiting,dyspnoea reduced urine output	DM,HT-12yrs, hypothyroidism-5yrs	febrile,dehydration,pallor+ PR-110/mt,BP-80/60mmHg, b/l pedal edema,b/l basal lung crepts,spo2-86%, L foot ulcer-non healing	9.3	19600	86%34%1%1%0%	26	224,000	76.9	26.7	31.3	CXRay-b/l basal lung infiltrates	Staphylococcus aureus	192	425	recovered
18	Lokesh	28	m	212/2970	27.4.17	fever,pain abdomen, vomiting,dyspnoea, reduced urine output	nil	febrile,dyspnoeic, BP-90/70mmHg,spo2-88% b/l basal lung crepts	12.9	13200	76%26%0%1%0%	18	83000	80.3	28.8	34.1	CXRay-b/lpleural effusion CT chest-b/l pleural effusion with collapse lung R>L,pl.fluid cytology reactive effusion,dengue IgM-positive	NG	48	50	recovered
19	Sathish kumar	27	m	212/85242	30.4.17	fever,chills&rigors, vomiting,cough,malena passage of dark coloured urine	nil	febrile,dehydrated,L eye congestion,PR-102/mt BP-100/60mmHg,b/lbasal lung crepts,epigastric tenderness	10.1	15100	82%30%0%0%1%	16	19000	72.3	25.1	32.3	CXRay-b/l lung infiltrates with consolidation, dengue IgM - positive	NG	24	84	recovered
20*	Puratchipathi	67	m	113/3855	10.6.17	fever,dyspnoea,vomiting cough with expectoration reduced urine output	nil	febrile,dyspnoeic,pallor+ PR-112/mt,BP-80/60mmHg, spo2-80%RA,tachypnoeic, RR-32/mt,b/l lung extensive crepts,abd.distension	8.6	22600	88%34%1%0%0%	25	110,000	77.1	23.3	30.3	USG abd-gross ascitis CT chest-R upper lobe consolidation,L upper lobe fibrosis	Pseudomonas aeruginosa	384	1180	expired
21	kamala kannan	28	m	124/3840	12.6.17	fever,vomiting,headache seizure1episode	seizure disorder-5yrs on irregular treatment	febrile,postictal drowsy confusional state, BP-100/70mmHg	12.6	13200	72%22%0%0%1%	18	232,000	88.8	29.2	32.9	-	NG	6	5.8	recovered
22	Mohanraj	37	m	124/85816	15.6.17	fever,headache,seizures1 episode,involuntary micturition after fits	seizure disorder-8yrs on irregular treatment chronic alcoholic,	febrile,postictal phase, PR-104/mt,dehydration+ tongue bite+ RS-added sounds+	12.1	12200	66%24%0%1%0%	12	115,000	85.9	27.9	32.4	-	NG	6	3	recovered
23	Gurunadhan	65	m	124/3700	18.6.17	fever,pain abdomen, inc.frequency of micturition	DM-4yrs,HT-4yrs on treatment	febrile,BP-90/60mmHg, dehydrated,PR-102/mt, suprapubic tenderness	12.6	15400	66%21%0%2%1%	14	232,000	89.2	32.4	36.1	urine RE-pus cells-10-15 urine c/s-growth+	NG	12	3.13	recovered
24	Sadaiyappan	35	m	223/3039	23.6.17	fever,pain abdomen, vomiting	chronic alcoholic	febrile,PR-108/mtBP-100/60 mmHg,dehydrated abd.-epigastric tenderness+	16.2	13600	76%25%1%0%1%	22	234,000	92.9	33.6	36.9	sr.amylase-372IU/L USGabd-pancreas obscured by bowel shadow,otherwise nothing significant	NG	12	54	recovered

25*	Poomani	55	f	121/84888	28.6.17	fever,swelling&pain L leg dyspnoea,abdominal distension	DM-1yr on treatment	febrile,drowsy,PR-110/mt, BP-80/60mmHg,spo2-92% L lung basal crepts,abd. Distension,L leg-edema+, warmth,tenderness,blebs+	10.2	21600	92%36%0%1%0%	26	262,000	77.2	28.6	32.1	CXRay-L minimal pleural effusion,USGabd-minimal ascitis,fatty liver	Staphylococcus aureus	192	568	recovered
26	Santhosh kumar	22	m	134/85393	29.6.17	fever,vomiting,myalgia, seizures 1 episode	seizure disorder-3yrs on irregular treatment	febrile,drowsy,PR-102/mt, BP-100/70mmHg	13.2	13200	74%24%0%0%1%	22	162,000	87.3	30.9	35.4	-	NG	6	3.2	recovered
27*	Sakthivel	52	m	213/87000	30.6.17	fever,swelling in perianal region with severe pain	DM-3yrs on treatment	febrile,BP-80/60mmHg, PR-114/mt,swelling perianal region burst open to ulcerative lesion with mucopurulent discharge, L inguinal lymphadenopathy, dehydrated	13	18600	86%36%0%1%0%	24	126,000	87.6	35.9	36.2	-	Staphylococcus aureus	192	482	recovered
28	Deepika	21	f	123/2278	30.6.17	fever,dyspnoea,reduced urine output	nil	febrile,PR-104/mt, BP-90/60mmHg,spo2-90%, L lung basal crepts,pallor+	8.5	13500	78%26%0%0%1%	20	24,000	76.2	26.2	32.6	CXRay-haziness L lower lobe,USG abd-minimal L pleural effusion,dengue IgM-positive	NG	24	86	recovered
29*	Yulandha mary	72	f	241/85904	2.7.17	fever,dyspnoea,reduced urine output,altered sensorium	DM-12yrs,HT-11YRS on treatment	febrile,not oriented, unconscious,pallor+, PR-120/mt,BP-80/60mmHg, spo2-85%,b/l lung basal crepts	8.4	21200	89%34%0%1%1%	26	262,000	74.2	25.4	31.2	CT chest- L lung consolidation,CT brain-multiple hypodense lesion both frontal/ parietal region, USGabd-b/l gr-1 MRD	Staphylococcus aureus	192	620	recovered
30	Selvam	54	m	134/87636	3.7.17	fever,vomiting,altered sensorium	DM-5yrs,HT-5yrs, seizure disorder-4yrs	febrile,unconscious,not oriented,BP-100/60mmHg, tachypnoeic,spo2-89% L lung basal crepts	10.2	12600	74%36%5%0%1%	18	269,000	77.6	29.3	32.7	Hyponatremia,CT brain-L -GC hypodensity	NG	12	9.8	recovered
31	Selva kumar	42	m	134/87686	3.7.17	fever,pain abd.,reduced urine output,abd. Distension,malena, hemetemesis	chronic alcoholic	febrile,hypotension,pallor+ dehydration,abd. Distension,BP-90/60mmHg	10.8	15,600	71%28%3%0%0%	16	80,000	74.6	29.7	32.3	USGabd-ascitis, hepatomegaly,dengue IgM-positive	NG	48	84	recovered
32	Dharani	34	f	123/84327	5.7.17	fever,pain abdomen, mild dyspnoea,vomiting	nil	febrile,PR-102/mt, BP-100/70mmHg,dehydration P/A-epigastric tenderness+	10.9	6800	76%21%0%1%0%	14	228,000	86.7	27.8	32.1	-	NG	12	3	recovered
33	Thangavel	60	m	134/87655	5.7.17	fever,reduced urine output,dysuria	DM-5yrs on treatment	febrile,dehydrated,pallor+ suprapubic tenderness+	8.5	12200	72%26%0%1%1%	16	168,000	77.8	26.2	32.3	urine-pus cells-8-10 urine c/s-growth+ CT abd- L-HUN	NG	24	4.5	recovered

34	Parvathy	70	f	121/87413	7.7.17	fever,vomiting,burning micturition	DM-3yrs on treatment	febrile,dehydrated,pallor+ P/A-diffuse tenderness+	9.6	13800	74%28%1%1%0%	14	268,000	78.5	25	31.7	USGabd-minimal free fluid in abdomen urine albumin+++ urine c/s-growth+	NG	12	76	recovered
35	Desigamani	41	m	134/2115	7.7.17	fever with chills,myalgia	nil	febrile,BP-90/60mmHg, dehydration, P/A-mild hepatomegaly+	13.5	11800	70%25%0%1%0%	16	80,000	83.4	28.4	34	USGabd-mild hepatomegaly, dengue IgM-positive	NG	24	94	recovered
36	Elumalai	55	m	113/45662	9.7.17	fever,vomiting,cough, dyspnoea	chronic alcoholic	febrile,dyspnoeic,RR-26/mt, BP-100/60mmHg,PR-104/mt, L lung basal crepts	11.2	10900	76%26%0%1%0%	22	122,000	89	28.7	32.1	sputum c/s-growth+ CXRay-haziness L lung lower lobe	NG	12	86	recovered
37	Bharathi	70	m	121/89398	12.7.17	fever,swelling with pain with ulcer 1st metatarsal region L foot,vomiting,dyspnoea	DM-7yrs,HT-6yrs,BA-8yrs on treatment	febrile,BP-100/70mmHg, PR-102/mt,ulcerative lesion L foot near 1st metatarsal region with serosanguinous discharge	10.2	13800	74%28%0%1%1%	16	257,000	81.6	26.2	32.7	pus c/s-growth+	NG	12	78	recovered
38	Ramesh	35	m	113/86023	13.7.17	fever,myalgia,cough	nil	febrile,dehydrated, BP-100/70mmHg,PR-102/mt, RS-b/l rhonchi+	13.2	11700	70%19%0%1%0%	18	215,000	83.7	29.3	35	-	NG	6	5.4	recovered
39	Kabir	32	m	134/87646	15.7.17	fever,vomiting,pain abd. Myalgia,malena	nil	febrile,BP-90/70mmHg, PR-106/mt,dehydration+, P/A-epigastric tenderness+ hepatomegaly,splenomegaly+	10.6	13200	70%24%0%0%0%	16	90,000	71.4	25.2	31.1	USG abd-mild hepatosplenomegaly UGIscopy-gastritis,dengue IgM-positive	NG	12	72	recovered
40	Sundaramoorthy	25	m	211/89604	16.7.17	fever,vomiting, arthralgia,dyspnoea,pain abdomen.	nil	febrile,PR-102/mt, BP-100/60mmHg,spo2-89%, RS-reduced breath sounds b/l lung base	11.6	12900	72%26%0%1%0%	16	48000	74.6	25.8	32.1	USGabd-b/l pleural effusion,GB wall edema, ascitis,CXRay-b/l pleural effusion,dengue IgM-positive	NG	24	110	recovered
41	Kamala	80	f	122/90369	17.7.17	fever, vomiting, altered sensorium	DM-5yrs, HT-4yrs on irregular treatment	febrile,drowsy,BP-170/100 mmHg,spo2-89% ,L lung basal crepts,dehydrated	10.2	14200	76%28%1%0%0%	20	198,000	78.6	26.8	32.8	CXRay-L pleural effusion MRI brain- age related changes	NG	12	76	recovered
42	Rakesh	21	m	211/89652	19.7.17	fever,arthralgia,vomiting	nil	febrile,dehydration, BP-90/60mmHg, L lung basal crepts,spo2-88%, RR-24/mt,	11.9	13800	74%26%0%1%0%	12	65000	82.1	27.2	32.2	USG abd-ascites,mild L pleural effusion, CXRay-mild L pleural effusion,dengue IgM-positive	NG	24	84	recovered

43	Prem kumar	65	m	241/89295	20.7.17	fever,pain abd,dysuria myalgia	DM 12yrs on treatment	febrile,BP-100/60mmHg, dehydration,suprapubic tenderness	10.9	14900	76%24%0%0%0%	14	262,000	84.2	28.6	32.6	urine-puscells 8-10, urine c/s-growth+	NG	12	12.8	recovered
44	Mani	60	m	241/43624	22.7.17	fever,altered sensoriun, seizures-1 episode,vomiting	chronic alcoholic, seizure disorder on irregular treatment	postictal phase,febrile, BP-100/70mmHg,dehydrated	11.1	13900	75%28%0%1%0%	12	265,000	85.1	29.2	33.1	CT brain-heterodense lesion in L temporoparietal region with specks of calcification seen	NG	6	3	recovered
45*	Egambaram	83	m	111/93435	25.7.17	fever,vomiting,pain abd, dysuria	DM-10yrs on treatment	febrile,dehydrated, BP-80/60mmHg,pallor+, suprapubic tenderness+	10.1	20200	86%36%0%0%0%	22	298,000	80.1	27.9	33.6	urineRE-pus cells-plenty urine albumin-3+ urine c/s-growth+	E.coli(ESBL)	192	856	recovered
46	Arumugam	56	m	111/96627	25.7.17	fever,cough,dyspnoea, vomiting,reduced urine output	PTB 2012-discontd treatment	febrile,dyspnoeic,RR-26/mt PR-104/mt, BP-100/60mmHg, spo2-89%, L basal lung crepts	11.2	13800	78%42%0%1%1%	22	166,000	81.2	31.2	32.6	CXRay-b/l lung infiltrates	NG	12	3.4	recovered
47	Rajendran	45	m	111/96556	26.7.17	fever,vomiting,myalgia, pain abd	nil	febrile,dehydrated,icteric BP-100/70mmHg, mild hepatomegaly	11.4	12100	76%28%0%0%1%	12	262,000	84.2	27.4	34.2	USG abd- mild hepatomegaly, elevated liver enzymes, lepto- positive 2+	NG	24	78	recovered
48	Moorthy	48	m	124/95827	27.7.17	fever with chills,myalgia	nil	febrile,dehydrated, BP-90/70mmHg, P/A-mild splenomegaly	12.2	12900	72%24%0%0%0%	14	256,000	82.2	29.2	33.7	USG abd- mild splenomegaly, P.S-P.vivax	NG	6	3.2	recovered
49	Thulasiammal	60	f	121/86522	28.7.17	fever,vomiting,pain abd, myalgia	HT-6yrs on treatment	febrile,dehydrated, BP-90/60mmHg,PR-104/mt, L lung basal crepts+, P/A- mild hepatomegaly	10.9	13000	76%29%0%1%1%	16	80,000	79.1	26.8	31.1	USG abd-mild hepatomegaly with ascitis,CXRay-L lung base infiltrates+,dengue IgM-Positive	NG	24	98	recovered
50	Murugan	56	m	225/106182	29.7.17	fever,myalgia,arthralgia, malena	HT-5yrs on treatment	febrile,dehydrated, BP-70/? on admission then BP-80/60mmHg after i.v fluids rashes over trunk+ pallor+	9.6	13900	78%26%0%1%0%	12	87000	78	25.6	31.8	USG abd-GB wall edema, dengue IgM-Positive	NG	12	112	recovered
51	Vijayakumari	28	f	224/106435	29.7.17	fever,headache,vomiting, myalgia, arthralgia	nil	febrile,BP-100/60mmHg,b/l ankle swelling,PR-102/mt	12.2	13800	72%24%0%0%0%	18	180,000	80.6	27.6	32.2	IgM Chikungunya-positive	NG	24	74	recovered
52	Madhavan	48	m	225/106656	30.7.17	fever,vomiting,altered sensorium	chronic alcoholic	febrile,drowsy,not oriented BP-90/70mmHg,PR-104/mt	12.9	12700	71%27%0%1%0%	12	230,000	86.2	27.8	32.3	hyponatremia	NG	6	3	recovered
53	Maragatham	23	f	224/106232	31.7.17	fever,vomiting,pain abd dysuria	hypothyroid on treatment	febrile, dehydrated, suprapubic tenderness	11.6	13600	76%22%0%0%1%	14	179,000	84.3	28.2	33.6	urine RE-pus cells 10-12 urine c/s-growth+	NG	12	3.2	recovered
54	Nagammal	60	f	122/107092	31.7.17	fever with chills,myalgia, vomiting,pain abdomen	HT-4yrs ,CAD on treatment	febrile,dehydrated, BP-90/60mmHg,PR-102/mt, P/A-epigastric tenderness+	12.4	12200	71%23%0%0%0%	10	220,000	81.9	27.2	31.3	-	NG	12	4.6	recovered

55	Sheikvalli	40	f	122/107442	3.8.17	fever,vomiting,pain abd, jaundice	HT-3yrs on treatment chronic alcoholic	febrile,dyspnoeic, BP-90/70mmHg,icteric,mild hepatomegaly	11.3	9800	78%26%0%0%0%	13	266,000	85.2	26.7	32.4	USG abd-mild hepatomegaly, elevated liver enzymes	NG	12	5.2	recovered
56	Fathima Banu	44	f	122/108058	4.8.17	fever,cough,dyspnoea	nil	febrile,BP-100/70mmHg,L lung basal crepts,PR-104/mt, RR-20/mt,spo2-90%	12.1	14900	78%36%0%1%0%	16	143,000	83.2	27	32.8	CXRay-L lung basal infiltrates	NG	24	5.6	recovered
57	Khan	23	m	221/116305	5.8.17	fever,vomiting,myalgia	nil	febrile,dehydrated, BP-90/70mmHg	11.9	13400	76%29%0%0%0%	11	231,000	84.6	27.7	32.9	-	NG	6	8.4	recovered
58	Chinnapappa	60	f	143/117203	12.8.17	fever,arthralgia,myalgia	DM- 8yrs on treatment	febrile,dehydrated,joint swelling and tenderness, BP-90/60mmHg	11.6	12900	73%28%0%1%0%	19	188,000	85.4	26.9	32.1	IgM chikungunya-positive	NG	12	96	recovered
59	Habeesha	48	f	121/118256	13.8.17	fever with chills, cough, dyspnoea, chest discomfort	COPD-4yrs on treatment,tobacco chewer-5yrs	febrile,dyspnoeic, PR-98/mt,BP-90/70mmHg, L basal crepts-lung,spo2-88%,RR-24/mt	10.3	14100	82%39%0%1%1%	21	234,000	78.9	27.1	30.2	CXRay-infiltrates L lung f/s/o chronic bronchitis	NG	24	78	recovered
60	Keerthika	35	f	121/107185	14.8.17	fever with chills &rigors, myalgia,vomiting	nil	febrile,dehydrated,pallor+ BP-100/70mmHg, splenomegaly, epigastric tenderness	9.1	12300	70%24%0%1%0%	12	190,000	76.8	25.2	31.9	USG abd-splenomegaly, P.S.-P.vivax	NG	6	10.2	recovered
61	Rose whilet	28	f	225/116286	16.8.17	fever with chills, myalgia, vomiting, pain abd	nil	febrile,dehydrated, BP-100/60mmHg,PR-102/mt, P/A-mild hepatomegaly	11.2	7800	71%20%0%0%1%	13	169,000	72.3	28.6	38.5	USG abd- mild hepatomegaly, IgM scrub typhus-positive	NG	12	104	recovered
62	Vijayakumari	42	f	121/109623	17.8.17	fever,myalgia,cough, dyspnoea	BA-6yrs on treatment	febrile,BP-90/60mmHg, PR-106/mt,dyspnoea, RR-26/mt,spo2-92%, RS-b/l rhonchi+	10.8	13700	77%32%0%1%0%	20	216,000	77.2	25	31.9	-	NG	6	10.1	recovered
63	Vasanthi	35	f	123/115960	19.8.17	fever with chills,pain abd,dysuria, vomiting	seizure disorder on treatment	febrile,dehydrated, BP-80/60mmHg,suprapubic tenderness+	11.7	15900	74%25%0%1%0%	12	146,000	86.2	29.5	33.6	urine pus cells-10-15 urine c/s-growth+	NG	12	9.2	recovered
64	Joynal	24	m	145/115675	19.8.17	fever,headache,1 episode fits,involuntary micturition,vomiting- 1 episode	seizure disorder on irregular treatment	febrile,postictal phase, PR-108/mt,spo2-94%,mild dyspnoea,RR-24/mt	12.6	11300	73%28%0%0%0%	15	245,000	86.6	28.6	34.2	-	NG	6	10.1	recovered
65	Shyamala	40	f	121/106884	22.8.17	fever,vomiting,headache, myalgia,pain abd	nil	febrile,dehydrated, BP-90/60mmHg,epigastric tenderness+	11.8	10800	72%22%0%1%0%	14	322,000	83.3	28.7	34.2	-	NG	6	9.6	recovered
66	Jeyanthi	37	f	225/115931	23.8.17	fever,myalgia,headache, malena	nil	febrile,BP-100/60mmHg, dehydrated,pallor+	9.6	10100	78%26%0%1%0%	18	96,000	73.2	24.6	32.4	IgM dengue-positive	NG	12	74	recovered
67	Kavitha	23	f	122/115853	25.8.17	fever,chills,myalgia, arthralgia,pain abd.	nil	febrile,dehydrated, swelling around ankle jt.	12.8	13900	80%32%1%0%1%	20	268,000	86.8	27.4	34.2	IgM chikungunya-positive	NG	12	86	recovered
68	Surya	28	f	224/115934	26.8.17	fever,myalgia,vomiting, pain abdomen	nil	febrile,dehydrated, BP-80/60mmHg,pallor+ P/A-epigastric tenderness+	9.2	12800	78%26%0%1%1%	17	170,000	79.8	24.2	32.1	UGIscopy-erosive gastritis	NG	12	92	recovered
69*	Vishwamithran	58	m	213/116767	27.8.17	fever,severe pain abdomen,swelling in abdomen with pain, vomiting,mild dyspnoea	megaloblastic anaemia,umblical hernia	febrile,PR-120/mt,pallor+ BP-80/60mmHg, spo2-90%, hernia-irreducible,abd.-tender,guarding,rigidity+ BS-sluggish,dehydrated, dyspnoea+	8.6	22600	92%42%0%1%1%	24	120,000	102.2	42.6	33.1	-	E.coli(ESBL)	384	1196	expired

70	Maruthu	40	m	113/131200	28.8.17	fever,vomiting,altered sensorium	nil	febrile,drowsy,dehydrated BP-90/70mmHg	11.6	12500	79%26%0%1%1%	19	280,000	80.2	27.9	34.8	Hyponatremia	NG	6	4.8	recovered
71	Sonu Thakul	25	m	225/116633	30.8.17	fever,chills & rigors, myalgia,headache	nil	febrile,dehydrated, BP-100/60mmHg,PR-102/mt	11.2	11700	76%32%1%0%0%	16	121,000	79.3	26.1	32.2	PS-P.vivax	NG	6	5.6	recovered
72	Sudha	28	f	121/106989	31.8.17	fever,myalgia,pain abd, vomiting	nil	febrile,dehydrated, BP-80/60mmHg,PR-104/mt	12.4	13900	78%29%0%1%0%	18	245,000	82.2	28.7	33.3	USG abd-subacute appendicitis	NG	12	24	recovered
73	Sathya	30	f	224/108118	2.9.17	fever,pain abd,vomiting, myalgia	nil	febrile,icteric,dehydrated, BP-90/70mmHg, P/A-epigastric tenderness+	11.5	12800	76%28%0%1%0%	16	189,000	77.4	25.6	31.6	lepto-positive2+	NG	12	50	recovered
74	Manibharathi	22	m	145/108387	3.9.17	fever,myalgia,cough, dyspnoea	BA-3yrs on treatment	febrile,dyspnoeic,RR-24/mt spo2-92%,BP-100/60mmHg, dehydrated,PR-108/mt.	12.8	13200	70%30%0%1%1%	22	287,000	83.2	27.8	33.7	-	NG	6	4.6	recovered
75	Nagaraj	38	m	134/122875	5.9.17	fever,myalgia,reduced oral intake	nil	febrile,dehydrated, BP-90/70mmHg,PR-102/mt	13.2	12600	76%24%0%1%0%	14	182,000	84.7	26	34.7	-	NG	12	3.13	recovered
76	Milanbanuri	30	m	113/122897	7.9.17	fever,pain abd,vomiting, myalgia	nil	febrile,dehydrated, PR-110/mt,BP-80/60mmHg, abd-tenderness R hypochondrium+guarding+	12.4	16300	78%28%0%0%0%	22	216,000	86.7	28.2	34.1	USG abd- GB wall thickening s/o cholecystitis	NG	96	86	recovered
77	Asha	22	f	121/122828	8.9.17	fever,myalgia,headache, bleeding gums	nil	febrile,dehydrated, BP-90/60mmHg,PR-106/mt	11.6	13600	78%32%0%1%0%	19	89,000	78.7	25.4	32.6	IgM dengue-positive	NG	12	72	recovered
78	Subbammal	60	f	241/122844	10.9.17	fever,dyspnoea,1 episode fits,involuntary micturition during fits	seizure disorder-10yrs on irregular treatment	febrile,drowsy,BP-100/70 mmHg, PR-104/mt,spo2-89% tongue bite+	11.8	12100	74%22%0%0%1%	18	240,000	80.8	26.4	32.8	-	NG	6	3	recovered
79	Sathish	24	m	134/121978	11.9.17	fever, with chills,myalgia, burning micturition, pain abdomen	nil	febrile,BP-100/60mmHg, PR-102/mt,dehydrated, P/A-suprapubic tenderness+	13.2	13800	78%36%0%1%0%	18	286,000	85.6	27.9	33.8	urine RE-pus cells10-15 urine c/s-growth+	NG	12	6.8	recovered
80	Dhanalakshmi	60	f	124/127215	13.9.17	fever,cough,dyspnoea, myalgia	COPD-9yrs on treatment	febrile,dyspnoeic,spo2-90% PR-110/mt,BP-110/70mmHg, RR-24/mt,RS-b/l rhonchi+	12.9	11900	76%32%0%1%0%	22	143,000	88.9	27.2	33.6	-	NG	6	7.3	recovered
81	Punitha	53	f	225/130396	14.9.17	fever,chills,myalgia, arthralgia,headache	nil	febrile,dehydrated, BP-100/70mmHg, swelling around ankle jt	12.8	13600	80%27%1%1%0%	16	280,000	87.8	27.6	34.9	IgM chikungunya-positive	NG	12	84	recovered
82	Raja	52	m	234/145602	16.9.17	fever,pain abd,vomiting	chronic alcoholic	febrile,BP-80/60mmHg, dehydration+,abd- epigastric tenderness,guarding , rigidity+	16.2	17800	82%43%0%0%0%	26	298,000	90.6	32.1	34.6	USG abd-s/o pancreatitis	NG	48	74	recovered
83	Suresh	43	m	113/131450	18.9.17	fever,myalgia, vomiting, headache	nil	febrile,dehydrated, BP-100/70mmHg,PR-104/mt	13.2	13200	74%32%1%0%0%	18	197,000	82.4	27.8	30.1	-	NG	6	3.13	recovered
84	Ranjith	41	m	224/141560	20.9.17	fever,myalgia,vomiting, reduced oral intake	HT-6yrs on treatment	febrile,dehydrated, BP-90/70mmHg,PR-102/mt, abd- epigastric tenderness+	12.6	14600	80%35%0%1%0%	21	120,000	89.6	33.2	35.2	scrub typhus Igm-positive	NG	12	48	recovered
85	Naveen	48	m	225/143682	22.9.17	fever,myalgia,malena, headache	nil	febrile,BP-90/60mmHg,eye congestion,dehydrated	13.9	12800	78%28%0%1%0%	18	90,000	82.6	28.4	32.4	IgM dengue-positive	NG	24	92	recovered
86	Amudha	42	f	224/139021	23.9.17	fever,myalgia,cough, dyspnoea	BA 6 yrs on treatment	febrile,PR-110/mt,spo2-90% RS-b/l rhonchi,RR-26/min	12.2	13800	76%22%0%1%0%	26	2,67,000	88.2	28.7	33.9	-	NG	6	9.4	recovered

87	Rajendra prasad	52	m	113/145778	26.9.17	fever, vomiting,pain abd, myalgia, hemetemesis	alcoholic,HT-4yrs on irregular treatment	febrile, dehydrated, PR-106/mt,BP-160/90mmHg epigastric tenderness-abd	10.4	12900	78%29%0%1%1%	24	1,65,000	76.4	24.3	30.4	UGIscopy-erosive gastritis	NG	12	96	recovered
88	Murali	35	m	134/146223	27.9.17	fever,malaise,vomiting, pain abd	nil	febrile,hypotension, dehydrated,icteric, P/A-mild hepatomegaly	13.6	13600	79%26%0%1%0%	18	2,63,000	82.4	27.3	33.1	lepto-positive3+ USG abd-mild hepatomegaly	NG	24	88	recovered
89	Geetha	62	f	223/149800	28.9.17	fever,vomiting,pain abd, myalgia	DM-12yrs on treatment	febrile,obese,dehydrated, PR-106/mt, BP-140/90mmHg	12.8	15600	82%32%0%1%1%	23	2,98,000	80.6	26.4	32.2	USG abd -s/o acute cholecystitis	NG	48	58	recovered
90	Kannan	28	m	145/148600	29.9.17	fever,headache,arthralgia, myalgia	nil	febrile,dehydrated, BP-90/70mmHg,PR-104/mt, swelling b/l ankle jt.	14.2	14900	77%28%0%1%0%	19	3,67,000	89.8	30.8	34.2	IgM chikungunya-positive	NG	12	82	recovered
91	Komala	35	f	212/149830	30.9.17	fever, myalgia, dysuria, pain abd, headache, inc. freq. of micturition	DM-4yrs on treatment	febrile, PR-112/mt, dehydrated,dyspnoeic, spo2-89%,irritable,RS-L basal crepts+,BP-100/70mmHg abd-suprapubic tenderness+	12.9	16800	82%32%0%1%1%	25	2,60,000	83.6	28.8	32.6	urine acetone-positive urineRE-albumin 2+ pus cells-12-15, urine c/s-growth+	NG	48	76	recovered
92*	Anbu	55	m	134/150271	2.10.17	fever,pain abd,vomiting, abd.distension,myalgia, reduced urine output, dyspnoea	chronic alcoholic, HT-7yrs on treatment	febrile,BP-110/mt, dehydrated,BP-106/76mmHg spo2-88%,abd-tense ascitis icteric,b/l PE,irritable	10.8	22900	88%42%1%0%1%	28	1,56,000	74.6	24.3	29.8	USG abd-ascitis, hepatomegaly	E.coli(ESBL)	384	1158	expired
93	Nalini	42	f	121/153890	3.10.17	fever,headache,myalgia, vomiting,pain abdomen	nil	febrile,dehydrated, BP-90/70mmHg, P/A-epigastric tenderness+	13.2	13700	78%26%0%1%0%	18	2,30,000	85.4	28.6	34.9	-	NG	6	3.13	recovered
94	Govindan	52	m	113/158901	5.10.17	fever,chills,myalgia, headache	nil	febrile,dehydrated, PR-104/mt,BP-100/70mmHg	11.8	12800	72%24%0%1%0%	16	2,98,000	80.6	26.1	32.8	PS-P.vivax	NG	6	3	recovered
95	Anandan	46	m	225/153489	6.10.17	fever,cough,dyspnoea, headache,vomiting	nil	febrile, PR-108/mt, dehydrated,dyspnoeic, RR-24/mt,spo2-92%, RS-scattered rhonchi+	13.6	12600	76%27%0%1%1%	18	1,85,000	84.3	34.6	34.2	-	NG	12	3.13	recovered
96	Vijayalakshmi	49	f	225/159806	8.10.17	fever,headache,myalgia, conjunctival congestion, vomiting	nil	febrile,BP-90/60mmHg, PR-106/mt,conjunctival congestion both eyes+, dehydrated	12.8	13800	78%30%0%2%1%	22	98,000	86.4	27.8	32	IgM dengue-positive	NG	12	92	recovered
97	muniyan	39	m	223/168961	11.10.17	fever,pain abd, vomiting, myalgia	alcoholic, smoker	febrile,PR-108/mt, BP-100/70mmHg, epigastric tenderness-abd, guarding+,dehydrated	16.4	15900	80%35%0%1%0%	24	1,63,000	90.2	34.6	34.1	USG abd-s/o Subacute pancreatitis	NG	24	32	recovered
98	Aruna	42	f	121/167654	12.10.17	fever,cough with expectoration,dyspnoea	nil	febrile,dyspnoeic, PR-106/mt,spo2-90%, BP-106/76mmHg, dehydrated	12.6	14800	82%42%0%1%1%	20	2,64,000	88.2	27.3	33.4	CXRay-infiltrates L lung sputum c/s-growth+	NG	24	56	recovered
99	Kandan	52	m	224/168650	13.10.17	fever,headache,myalgia, vomiting	nil	febrile,BP-100/60mmHg, dehydrated	11.5	13100	78%25%0%1%0%	18	95,000	79.5	24.3	31.6	IgM dengue-positive	NG	12	96	recovered
100	Krishnaveni	42	f	123/169321	15.10.17	fever,dysuria,pain abd, myalgia	DM 8yrs on treatment	febrile,BP-100/70mmHg, abd-suprapubic tenderness, PR-102/mt	12.1	14800	79%28%0%1%0%	16	2,77,000	80.9	26	32.4	urineRE-pus cells 10-15 urine c/s-growth+	NG	24	14.6	recovered

101	Karuppan	52	m	113/166932	16.10.17	fever,vomiting,myalgia, reduced oral intake	alcoholic	febrile,icteric,PR-108/mt, abd-hepatomegaly, dehydrated	11	13900	75%26%0%1%0%	22	1,46,000	78.3	25.4	31.1	elevated liver enzymes USG abd-hepatomegaly, s/o hepatitis	NG	48	78	recovered
102	Ramasamy	44	m	145/166946	2.11.17	fever,myalgia,headache, arthralgia	HT-5yrs on treatment	febrile, dehydrated, BP-90/60mmHg,b/l ankle swelling+warmth+ tenderness+	13.6	13200	74%27%0%1%0%	17	2,24,000	88.6	28.5	33.8	IgM chikungunya-positive	NG	12	82	recovered
103	Hari	48	m	134/167352	8.11.17	fever,headache,cough,fits 1 episode	seizure disorder-skipped regular medicines-15 days	febrile,postictal phase, PR-110/mt,BP-106/66mmHg, tongue bite+	14.3	12800	72%22%0%0%0%	19	1,98,000	92.1	34.2	35.4	-	NG	6	3.4	recovered
104	Ramya	32	f	123/167785	10.11.17	fever,myalgia,vomiting, pain abd	nil	febrile,dehydrated, BP-100/70mmHg	13.4	13600	70%25%0%1%1%	18	1,20,000	84.2	27.6	32.4	IgM scrub typhus-positive	NG	12	96	recovered
105	Ramesh	45	m	225/168501	12.11.17	fever,vomiting,pain abdomen,giddiness	nil	febrile,PR-106/mt,BP-90/70 mmHg,dehydrated,P/A-guarding+,rigidity+, tenderness R iliac region+	11.8	16100	82%40%0%1%0%	22	2,10,000	86.2	29.4	34.1	USG abdomen-F/S/O acute appendicitis	NG	48	72	recovered
106	Keerthana	46	f	121/169002	15.11.17	fever,reduced oral intake, vomiting,myalgia,pain abd	nil	febrile,icteric,BP-100/70 mmHg,PR-102/mt,P/A-hepatomegaly+	10.9	12100	74%29%0%1%0%	18	1,98,000	89.4	28.6	33.2	Lepto-positive3+ USG abd-Hepatomegaly	NG	24	98	recovered
107	Radhika	42	f	123/170910	17.11.17	fever with rigors,myalgia, headache,reduced oral intake,vomiting-1 episode	Hypothyroid on treatment since 4yrs	febrile,dehydrated,BP-96/70 mmHg,PR-106/mt,P/A-splenomegaly	10.3	14500	78%28%0%1%0%	17	2,40,000	78.2	24.9	32.1	P.S-P.vivax USG abd-splenomegaly	NG	12	3.1	recovered
108	Ravi	30	m	111/171231	20.11.17	fever,vomiting,pain abd, weakness both lower limbs	alcoholic,smoker	febrile,dehydrated,BP-100/70 mmHg,PR-102/mt,P/A-soft epigastric tenderness+ B/L lower limb-tone,power reduced,plantar reflex-normal	11.6	12300	74%26%0%0%1%	19	2,26,000	82.8	26.4	33.5	Hypokalemia	NG	6	3.6	recovered
109	Aruna	45	f	122/173456	25.11.18	fever with chills,myalgia, pain lower abdomen	DM-3yrs on treatment	febrile,PR-106/mt,BP-100/70 mmHg,P/A-suprapubic tenderness+	12.6	13800	78%28%0%1%0%	18	1,96,000	84.6	27.2	32.8	urine RE-10-15pus cells+ urine C/S-growth+	NG	12	9.8	recovered
110	Azhagu	35	f	121/173960	26.11.18	fever,cough with expectoration,dyspnoea, reduced oral intake	BA on treatment -7yrs	febrile,PR-112/mt,BP-100/70 mmHg,RS-B/L rhonchi,L basal crepts+dehydrated	11.4	14200	80%28%0%1%0%	22	2,16,000	78.9	24.2	31.4	CXR-F/s/o chronic bronchitis	NG	12	74	recovered
111	Poongodi	43	f	123/176904	26.11.18	fever,altered sensorium, dyspnoea,vomiting-recurrent,reduced oral intake,bladder incontinence	DM-10yrs on irregular treatment	febrile,PR-120/mt,BP-90/60 mmHg,dehydrated,spo2-89% drowsy,disoriented,dyspnoeic CVS/RS-NAD,P/A-soft,power tone both upper and lower limbs -normal,plantar reflex-normal.	12.6	13100	74%26%0%0%0%	14	2,26,000	82.4	27.6	34.2	High blood sugar levels ABG analysis showed f/o acidosis urine acetone-positive Hyponatremia	NG	24	106	recovered
112*	Rathnam	69	m	212/178120	27.11.18	fever,dyspnoea,cough, reduced urine output swelling both legs	DM-12yrs on treatment smoker,alcoholic	febrile,BP-90/60mmHg,PR-110/mt,RS-L basal crepts, b/l pedal edema+,RR-28/mt	10.2	23800	82%38%0%1%0%	24	1,90,000	78.6	24.2	30.4	CXR-infiltrate L lung,f/s/o consolidation	Staphylococcus aureus	192	472	recovered

113	Govindraj	45	m	113/178291	27.11.18	fever,vomiting,headache, myalgia,pain abd	nil	febrile,PR-102/mt,BP-80/60 mmHg,RS-b/l basal crepts	12.6	13400	80%22%0%1%0%	18	92,000	79.4	26.8	32.1	CXR-b/l lung infitrates IgM dengue-positive	NG	24	72	recovered
114	Arul	32	m	113/179231	28.11.18	fever,myalgia,arthralgia, vomiting	nil	febrile,BP-100/60mmHg,PR-104/mt,CVS/RS-NAD,P/A-soft.	11.4	14200	81%24%0%1%0%	16	1,56,000	88.6	27.4	31.4	IgM Chikungunya-positive	NG	12	84	recovered
115	kannan	62	m	124/179432	29.11.18	fever,altered sensorium, vomiting,fits-1 episode, urinary incontinence+, dyspnoea	alcoholic-10yrs, seizure disorder-6yrs on irregular treatment	febrile,BP-100/70mmHg,PR-102/mt,drowsy,spo2-88% RS-added sounds,CVS-NAD, P/A-soft	12.2	13800	78%26%0%0%0%	20	1,98,000	82.6	26.4	32.6	hyponatremia,CT brain-L -GC hypodensity	NG	6	4.6	recovered
116	Nallathambi	42	m	123/179542	30.11.18	fever,dysuria,pain abdomen,myalgia, fever associated with chills and rigors	DM-10yrs on irregular treatment	febrile,BP-90/60mmHg,PR-106/mt,P/A-suprapubic tenderness+,dehydrated	12.4	15200	84%28%0%1%0%	18	2,60,000	84.2	27.4	34.2	urine RE-10-15pus cells+ urine C/S-growth+	NG	12	3.4	recovered
117	Gokul	35	m	113/179641	30.11.18	fever with chills and rigors, myalgia, vomiting	nil	febrile,BP-100/70mmHg, PR-102/mt,CVS/RS-NAD, P/A-splenomegaly+,CNS-NAD	11.8	13100	82%27%1%0%0%	18	1,50,000	82.2	26.8	32.2	PS-P.vivax USG abd-mild splenomegaly	NG	12	4.2	recovered
118	Kaviyarasan	28	m	125/179952	5.12.18	fever,cough,dyspnoea, reduced oral intake	smoker BA since 6yrs	febrile,dyspnoeic,spo2-91%, PR-110/mt,RS-L basal crepts, scattered rhonchi+	12.2	12500	86%29%0%1%0%	20	2,10,000	85.8	28.4	34.6	CXR-bronchitis with consolidation L lung	NG	12	60	recovered
119	Durga	29	f	121/181204	10.12.18	fever,pain abdomen, vomiting,giddiness	nil	febrile,BP--90/60mmHg, PR-102/mt,dehydrated, P/A-tenderness R hypochondrium+ guarding+BS+	11.4	13200	82%28%1%0%0%	16	1,90,000	82.4	27.6	33.2	USG abd-s/o acute cholecystitis	NG	48	82	recovered
120	Balu	56	m	113/183621	15.12.18	fever,dyspnoea,cough reduced urine output, fever associated with chills chest pain	smoker-10yrs,	febrile,dehydrated, dyspnoeic,spo2-90%,RR-28/mt, RS-L basal crepts +	12.6	14800	86%29%0%1%0%	20	1,80,000	88.2	25.6	32.4	CXR-L lung minimal pleural effusion+ sputum c/s-growth+	NG	24	74	recovered
**121	Chandran	45	m	123/184562	19.12.18	fever,with chills, vomiting, myalgia,pain abdomen, dyspnoea	nil	febrile,dehydrated,dyspnoeic PR-108/mt,BP-90/60mmHg RS-L basal crepts,spo2-89%, RR-36/mt	11.2	15600	88%30%0%1%0%	22	69,000	78.2	23.6	31.4	CXR-L lung minimal pleural effusion+, USG abd-GB wall edema+ minimal ascitis+ IgM dengue-positive	NG	96	110.2	expired
122	Kavitha	28	f	121/186234	26.12.18	fever,cough,dyspnoea, vomiting,reduced oral intake,myalgia	nil	febrile,dyspnoeic,PR-100/mt, BP-100/60mmHg,RR-28/mt, RS-b/l rhonchi+,L basal crepts	12.6	18200	87%28%0%1%1%	24	1,89,000	84.6	28.4	33.2	CXR-b/l lung infitrates+ sputum c/s-growth+	NG	12	46	recovered
123	Anand	42	m	125/187635	28.12.18	fever,myalgia,reuced oral intake,vomiting+	nil	febrile,BP-90/60mmHg,PR-98/mt,CVS/RS-NAD,P/A-epigastric tenderness+ mild splenomegaly	11.6	13600	82%26%1%0%0%	18	1,20,000	82.4	26.6	32.4	IgM scrub typhus-positive	NG	12	98	recovered

124	Devi	42	f	121/188652	2.1.18	fever,with chills,pain lower abdomen,myalgia	DM -7yrs on treatment	febrile,dehydrated,PR-102/mt BP-100/70mmHg,P/A-soft, suprapubic tenderness+	10.8	12800	81%24%0%1%0%	16	1,96,000	76.8	26.2	31.8	urine RE-pus cells 10-15 urine c/s-growth+	NG	24	4.2	recovered
125	Arul	42	m	124/189652	6.1.18	fever,altered sensorium, urinary incontinence, fits-1 episode,vomiting	alcoholic,seizure disorder on irregular treatment	febrile,dyspnoeic,PR-110/mt BP-160/100mmHg,RR-38/mt spo2-88%,RS-added sounds tongue bite+	11.8	14600	82%26%0%1%0%	20	1,72,000	86.6	27.6	32.8	CT brain-heterodense lesion in R temporoparietal region with specks of calcification seen	NG	6	3.6	recovered
126	Vigneswaran	32	m	223/189832	10.1.18	fever,scrotal swelling L, pain,myalgia	DM-4yrs on treatment	febrile,L scrotal edema+ L inguinal lymphadenopathy dehydrated,BP-100/70mmHg	12.6	15400	88%28%1%1%0%	18	2,20,000	82.4	28.2	32.2	I &D done and pus c/s-growth+	NG	24	72	recovered
127	Mohan	36	m	234/192983	12.1.18	fever,hematuria,pain abdomen,vomiting, reduced urine output	nil	febrile,dehydrated,BP-100/70 mmHg, PR-102/mt,P/A-diffuse tenderness+no guarding/rigidity	13.2	16400	86%24%0%0%0%	14	1,86,000	84.6	31.2	34.2	urine RE-pus cells+RBC+ albumin+++ USG abd-s/o acute glomerulonephritis	NG	24	120.6	recovered
128	Gandhi	32	m	256/194328	14.1.18	fever,severe pain abdomen vomiting,reduced oral intake,myalgia	nil	febrile,PR-106/mt,BP-80/60 mmHg,P/A-tenderness R iliac region, guarding+rigidity+	12.6	13600	88%28%0%0%0%	12	2,60,000	82.2	26.8	32.6	USG abd-s/o acute appendicitis	NG	96	82	recovered
129	Anitha	42	f	121/195692	15.1.18	fever,arthralgia,myalgia, vomiting	nil	febrile,dehydrated, BP-100/70mmHg,PR-102/mt b/l ankle edema+,swelling smaller joints of hands	11.8	13800	82%24%0%1%0%	16	1.92,000	84.6	27.2	34.4	IgM chikungunya-positive	NG	12	86	recovered
130	Karunanithi	56	m	235/196783	17.1.18	fever,pain abdomen, vomiting,myalgia,h/o passage of high coloured urine,h/o malena	alcoholic, DM-5yrs on irregular treatment	febrile,BP-90/60mmHg, PR-108/mt,icterus+ pallor+,P/A-hepatomegaly+ tenderness R hypochondrium	9.6	15800	88%28%0%1%0%	22	1,76,000	76.5	24.6	30.2	USG abd-hepatomegaly gallstones+ f/s/o-obstructive jaundice	NG	24	98	recovered
131	Palani	45	m	124/197832	18.1.18	fever,with chills and rigors myalgia,headache	nil	febrile,dehydrated, BP-100/70mmHg,PR-102/mt P/A-mild splenomegaly,	11.2	13200	84%26%1%0%0%	12	2,26,000	80.2	26.6	32.2	USG abd-mild splenomegaly PS-P.vivax	NG	12	10.1	recovered
132	Sakthi	28	f	111/198372	20.1.18	fever,pain abdomen, myalgia,vomiting	nil	febrile,dehydrated,icteric, BP-110/70mmHg,PR-106/mt P/A-hepatomegaly+	12.6	14100	86%26%0%1%0%	14	1,98,000	82.6	28.6	33.2	USG abd-mild hepatomegaly lepto positive 2+	NG	12	72	recovered
133	Arunai	53	f	213/199764	22.1.18	h/o unknown bite L foot swelling at local site,pain+ h/o fever+	nil	febrile,BP-90/60mmHg, PR-108/mt,RR-22/mt, swelling ,redness at local site L foot,no obvious bite mark, drowsy,CVS/RS-NAD,P/A-soft.	12.4	13200	84%24%0%1%0%	16	1,50,000	84.6	27.6	32.4	I&D done,pus c/s-growth+,BT/CT-normal	NG	48	78	recovered
134	Latha	32	f	121/199872	24.1.18	fever,dysuria,reduced urine output, pain abd,	DM since 4yrs on treatment	febrile,BP-90/60mmHg, PR-104/mt,dehydrated, P/A-diffuse tenderness+	10.9	15600	82%26%0%1%0%	18	2,60,000	78.4	24.6	31.2	elevated renal parametes urine RE-albumin++, pus cells-10-15 urinec/s-growth+	NG	12	60	recovered

135*	Ravi	62	m	111/218926	26.1.18	fever,altered sensorium dyspnoea,cough, chest pain,sweating, myalgia	HT-since 10yrs on treatment,DM-since 5yrs on irregular treatment	febrile,drowsy,dyspnoeic spo2-89%,PR-110/mt, BP-86/60mmHg,CVS-NAD, RS-L basal crepts+B/L rhonchi pallor+,dehydrated	9.6	20600	88%32%1%1%0%	22	1,20,000	75.2	25.4	30.6	CXR-f/s/o consolidation L lung with moderate pleural effusion	Staphyloccus aureus	192	730	recovered
136	Punitha	54	f	121/238920	27.1.18	fever with chills,pain abdomen,dysuria, vomiting	DM since 5 yrs on treatment	febrile,PR-102/mt,BP-80/60 mmHg,dehydration+ P/A-soft,suprapubic tenderness+	11.2	13800	80%24%0%1%0%	16	1,90,000	85.6	26.4	32.8	urine RE albumin-2+ pus cells-10-15 urine C/S-growth+	NG	12	54	recovered
137	Saranya	35	f	125/238981	30.1.18	fever,severe arthralgia, myalgia,vomiting	nil	febrile, BP-90/60mmHg, PR-98/mt,swelling B/L ankle, smaller joints of hands, CVS/RS-NAD,P/A-soft, CNS-NAD	10.6	12900	82%22%1%0%0%	20	2,68,000	79.6	24.8	33.6	IgM chikungunya-positive	NG	24	45	recovered
138	Govindan	45	m	111/239726	31.1.18	fever,vomiting,pain abdomen,h/o passing high coloured urine, reduced oral intake, myalgia	nil	febrile,icteric,dehydrated,PR-96/mt,BP-96/60mmHg, P/A-mild hepatomegaly	12.8	14600	78%29%0%1%0%	18	1,96,000	88.6	28.4	34.6	Leptospirosis-3+positive	NG	24	76	recovered
139	Kavitha	48	f	235/239982	2.2.18	fever with chills,ulcer L foot 3days, severe pain L foot associated with swelling, h/o trauma L foot	DM since 8yrs on irregular treatment	febrile,BP-80/60mmHg, PR-102/mt,ulcer L foot with serosanguinous discharge,L inguinal lymphadenopathy	11.6	15600	82%32%0%1%0%	24	2,16,000	80.6	26.8	32.6	pus c/s-growth+	NG	12	52	recovered
140*	Periyasamy	56	m	206/242019	3.2.18	fever,dyspnoea,chest discomfort,vomiting, sweating,restlessness, reduced oral intake	smoker, alcoholic DM since 10yrs on irregular treatment	febrile,restless,dyspnoeic, spo2-88%,PR-108/mt, BP-190/100mmHg,RS-b/l basal crepts,CVS-NAD, P/A-hepatomegaly	12.6	21900	89%36%0%1%1%	26	1,98,000	82.6	28.6	31.8	CXR-b/l lung infiltrates USG abd-hepatomegaly Elevated liver enzymes.	Staphyloccus aureus	192	800	recovered
141	Karuppusamy	62	m	145/243217	5.2.18	fever,cough with expectoration, hemoptysis,chest pain, dyspnoea	chronic smoker	febrile,BP-100/70mmHg, PR-106/mt,dyspnoeic,spo2-89%,RS-b/l extensive rhonchi pallor+	9.8	14800	86%34%0%1%1%	26	2,56,000	74.6	24.5	29.6	CXR-f/s/o chronic bronchitis	NG	12	48	recovered
142	Sekar	35	m	224/24467	6.2.18	fever,severe pain abdomen, vomiting,headache, myalgia	nil	febrile, BP-80/60mmHg, PR-104/mt,P/A-tenderness R iliac fossa,guarding, rigidity+dehydration+	10.6	18200	88%36%0%1%0%	24	1,88,000	79.4	25.6	30.6	USG abd-s/o acute appendicitis.	NG	24	96	recovered
143	Kamala	46	m	232/24486	7.2.18	fever,vomiting,myalgia, pain abdomen,reduced oral intake	DM since 6yrs on treatment	febrile,BP-90/60mmHg, PR-98/mt,dehydrated,obese P/A-tenderness R hypochondrium+	11.8	15800	86%32%0%0%1%	18	2,26,000	84.6	28.2	33.8	USG abd-s/o acute cholecystitis	NG	24	32	recovered

144*	Abdul basha	56	m	125/24578	8.2.18	fever,dyspnoea,cough, reduced urine output swelling both legs,reduced oral intake,	DM-since 12 yrs on irregular treatment, chronic smoker, alcoholic	febrile,dyspnoeic,spo2-88%, b/l pedal edema+pallor+ BP-150/100MMHg,PR-106/ mt,RR-28/mt,CVS-NAD RS-L basal crepts, P/A-hepatomegaly	10.4	23600	89%28%0%1%0%	24	1,88,000	78.6	25.4	30.6	CXR-L pleural effusion USG abd-hepatomegaly	Acinetobacter spp.	192	782	recovered
145	Veerasamy	48	m	236/24589	9.2.18	fever,vomiting, hemetemesis,pain abdomen reduced oral intake	chronic alcoholic	febrile,dehydrated,pallor+ icteric,P/A-hepatomegaly, tenderness epigastric region+	9.4	14900	84%32%1%0%0%	22	2,65,000	76.8	24.4	31.6	USG abd-hepatomegaly UGIscopy-erosive gastritis	NG	12	54	recovered
146	Gurusamy	52	m	145/24687	11.2.18	fever,altered sensorium, fits 1 episode ,vomiting, involuntary micturition	chronic alcoholic, chronic smoker, seizure disorder on irregular treatment	febrile,drowsy,BP-140/100 mmHg,PR-108/mt,spo2-89% RS-added sounds, tongue bite+	11.2	12600	82%31%0%0%1%	18	2,42,000	78.2	26.4	32.6	hyponatremia, CT brain-hypodense lesion in L parieto temporal region	NG	6	11	recovered
147	Paneer	48	m	212/24738	13.2.18	fever,dyspnoea,myalgia, bleeding gums,cough	nil	febrile,BP-80/60mmHg, PR-108/mt,pallor+dyspnoeic spo02-88%,drowsy,RS-b/l basal crepts	10.2	15700	86%34%0%1%0%	26	90,000	77.4	26.8	31.8	CXR-b/l lung infitrates IgM dengue-positive	NG	24	42	recovered
148	Gopal	44	m	124/24892	17.2.18	fever with chills and rigors myalgia,vomiting	nil	febrile,BP-90/60mmHg, PR-106/mt,P/A-splenomegaly dehydrated	12.8	13800	82%28%0%1%0%	18	2,64,000	82.8	27.2	33.4	PS-P.vivax +	NG	6	36	recovered
**149	Mani	35	m	212/24902	21.2.18	fever,vomiting,headache, myalgia,pain abdomen, cough,dyspnoea	nil	febrile,dehydrated, dyspnoeic,spo2-90%, BP-80/60mmHg,PR-98/mt, P/A-hepatomegaly, RS-L basal crepts,	12.4	12100	80%32%0%1%0%	22	86,000	84.8	29.4	34.6	USG abd-hepatomegaly CXR-minimal L pleural effusion, IgM dengue-positive	NG	96	120.6	expired
150	Saravanan	56	m	235/25679	27.2.18	fever,vomiting,pain abdomen,vomiting, hemetemesis,reduced oral intake	chronic alcoholic	febrile,dehydrated,BP-90/60 mmHg,PR-102/mt,icteric, P/A-hepatomegaly, tenderness epigastric region+	11.6	14800	86%34%0%1%0%	24	1,98,000	84.8	27.6	32.4	USG abd-hepatomegaly UGI scopy-erosive gastritis	NG	12	58	recovered

*** * CULTURE NEGATIVE CASES**

Sample no	BHI Broth	Culture media	Gram stain	Catalase test	coagulase test	Oxidase test	motility	Indole	TSI	Citrate	Urease	Identification	Antibiogram								VSA /MIC	Outcome	CRP (mg/l)	IL-6 (pg/ml)	PCR(gene detected)
*17	turbid	Mac-LF opaque colonies	GPCclusters	positive	slide/tube positive	-	-	-	A/A	-	-	Staphylococcus aureus(MSSA)	cip-R	Pen-s	cot-s	LZ-s	Tet-s	CX-s	Ery-s	-	VAN s	recovered	192	425	-
*20	turbid, lysis+	Mac-NLF colonies	GNB	positive	-	positive	motile	-	K/NC	positive	-	Pseudomonas aeruginosa	Ak-s	Pt-s	Gm-s	cip-s	Imp-s	CAZ-R	-	-		expired	384	1180	-
*25	turbid	Mac-LF opaque colonies	GPCclusters	positive	slide/tube positive	-	-	-	A/A	-	-	Staphylococcus aureus(MSSA)	cip-s	Pen-s	Ery-s	LZ-s	Tet-s	CX-s	cot-R	-	VAN s	recovered	192	568	-
*27	turbid	Mac-LF opaque colonies	GPCclusters	positive	slide/tube positive	-	-	-	A/A	-	-	Staphylococcus aureus(MSSA)	cip-R	Pen-s	LZ-s	CX-s	Tet-s	Ery-s	cot-R	-	VAN s	recovered	192	482	-
*29	turbid	Mac-LF opaque colonies	GPCclusters	positive	slide/tube positive	-	-	-	A/A	-	-	Staphylococcus aureus(MSSA)	Pen-s	Ery-s	LZ-s	Tet-R	CX-s	cip-R	cot-R	-	VAN s	recovered	192	620	-
*45	turbid	Mac-LF colonies	GNB	positive	-	-	motile	Positive	A/A	-	-	E.coli(ESBL)	Ak-s	ctx-R	Gm-R	cip-R	cot-R	Amp-R	Tet-s	-		recovered	384	856	CTX-M, TEM
*69	turbid	Mac-LF colonies	GNB	positive	-	-	motile	Positive	A/A	-	-	E.coli(ESBL)	Ak-s	Gm-s	Ctx-R	cip-R	cot-R	Tet-s	Amp-R	-		expired	384	1196	CTX-M, TEM
*92	turbid, lysis+	Mac-LF colonies	GNB	positive	-	-	motile	Positive	A/A	-	-	E.coli(ESBL)	Ak-s	Gm-R	Ctx-R	cip-R	cot-R	Amp-R	Tet-s	-		expired	384	1158	CTX-M, TEM
*112	turbid	Mac-LF opaque colonies	GPCclusters	positive	slide/tube positive	-	-	-	A/A	-	-	Staphylococcus aureus(MSSA)	cip-s	Ery-s	Pen-S	LZ-s	tet-s	cot-R	CX-s	-	VAN s	recovered	192	472	-
*135	turbid	Mac-LF Opaque colonies	GPCclusters	positive	slide/tube positive	-	-	-	A/A	-	-	Staphylococcus aureus(MSSA)	Ery-s	Pen-s	cot-s	cip-R	LZ-s	Tet-s	CX-s	-	VAN s	recovered	384	730	-
*140	turbid	Mac-LF Opaque colonies	GPCclusters	positive	slide/tube positive	-	-	-	A/A	-	-	Staphylococcus aureus(MRSA)	LZ-s	cip-s	Ery-s	cot-R	Tet-R	Pen-R	CX-R	-	VAN s	recovered	192	800	mecA
*144	turbid, lysis+	Mac-NLF colonies	GNB	positive	-	-	non-motile	-	K/NC	Positive	-	Acinetobacter spp.	Pt-s	Tet-s	Ak-R	Gm-R	cip-R	CAZ-R	Imp-s	cot-R		recovered	384	782	-

*** CULTURE POSITIVE CASES**